Original Article

Study of hesperetin effect on modulating transcription levels of MLH1 and MSH2 genes in SKBR3 breast cancer cell line

Naser Hameed Saleh, Ahmed Salim Kadhim Al-Khafaji[1], Esmaeil Babaei

Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran, [1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

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Abstract

Hesperetin (HSP), a flavonoid, has been validated to modify gene expression and function as an epigenetic agent to stop the development of breast carcinoma cells. HSP was investigated in this research to evaluate the expression of the MLH1 and MSH2 genes in cancerous breast cell lines (SKBR3) and healthy cell lines (MCF-11A) after exposure to different dosages (200, 400, and 600 μM/mL) of HSP. After 48 h of exposure, SKBR3's half-maximal inhibitory concentration was 289.6 μM/mL and MCF-10A's was 855.4 μM/mL. The research found that increasing HSP concentrations were closely correlated with an increase in MLH1 gene levels in the SKBR3 cell line, as shown by median and percentile values. HSP therapy caused the MLH1 gene expression to substantially vary in different groups, and in the SKBR3 cell line, MSH2 gene expressions were elevated in a dose-escalating manner. Moreover, HSP also raised the number of apoptotic cells, with the fraction of apoptotic cells escalating substantially at doses of 400 and 600 μM/mL. The outcomes suggested that HSP has the potential to be utilized as a therapeutic intervention for breast cancer, as it can induce apoptosis and reduce cell viability.

Key words: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test, apoptosis, cytotoxicity, flow cytometry, half-maximal inhibitory concentration 50, hesperetin, malignancy, MLH1 gene expression, MSH2 gene expression

INTRODUCTION

People all across the globe are affected by breast cancer, a complex, multidimensional condition. Due to the disease's molecular heterogeneity, new treatment alternatives have emerged, with an emphasis on more biologically targeted drugs and therapy de-escalation to reduce side effects. Early breast cancer is anticipated to be curable because of improvements in multimodal therapy, which have increased patient cure rates to 80%.[1-3] Advanced breast cancer may still be treated, and the major objectives of treatment are to preserve or enhance the quality of life while reducing side effects and managing symptoms. Locoregional therapy and systemic therapy are the two main pillars of breast cancer treatment, with the disease's histology and molecular features having a substantial influence on available alternatives. There are four distinct forms of breast carcinoma, according to Perou and Sorlie's intrinsic categorization: luminal A and luminal B, basal-like, and HER2-enriched.[4,5]

Address for correspondence:
Prof. Dr. Esmaeil Babaei,
Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.
E-mail: babaei@tabrizu.ac.ir

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Several genes are correlated with breast carcinoma. The potency of abnormal gene expression as a potential biomarker has recently been interrogated for malignance-related survival in breast cancer patients[6,7] as well as other carcinomas.[7-9] The first two genes are BRCA1 and BRCA2, which act as tumor suppressor proteins and are located in chromosomes 17q21 and 13q12, respectively.[10,11] Any BRCA1 defect may result in apoptosis and aberrant cell proliferation. Contrarily, BRCA2 has a role in controlling the recombinational repair of DNA double-strand breaks.[12] Breast cancer risk may be greatly raised by BRCA1 and BRCA2 mutations.[13] Another gene is HER2, which is an oncogene protein and a tumor marker that may be found on chromosome 17q12. The epidermal growth factor signaling pathway is activated by the tyrosine kinase receptor family member HER2.[14] About 20% of individuals diagnosed with early-stage breast carcinoma have HER2 overexpression, which is linked to poor clinical outcomes.[15]

The range of breast cancer treatments has expanded, and being knowledgeable about these alternatives can assist doctors in better managing the patients' care. The staging of tumor-node-metastases, lymphatic dissemination, histologic grade, hormone receptor status, ERBB2 overexpression, comorbidities, patient age, and menopausal status are often used to evaluate the prognosis and treatment choices for breast cancer.[16,17] In nonmetastatic breast cancers, the best action could be eradicating the whole tumor from the patient to prevent metastasis recurrence. Local therapy, like surgical resection, axillary lymph node removal, and postoperative radiation, could also be considered.[18] Based on the breast cancer subtype, systemic treatment, such as neoadjuvant, adjuvant, or both, may be delivered. The main systemic therapy for HR+/ERBB2+ breast cancer is endocrine therapy.[19,20]

Citrus fruits contain hesperidin, a flavonoid that undergoes enzymatic hydrolysis in the digestive system to produce hesperetin (HSP).[21-23] The anticancer abilities of HSP and its impacts on molecular disease pathways have been the subject of recent research.[24] HER2-positive breast cancer, in which the HER2 receptor protein is overexpressed and has unregulated tyrosine kinase activity, is one of the most aggressive and poorly prognostic subgroups of the disease.[25] Chemotherapy and immunotherapy are two treatment options for HER2-positive malignancies, but both have side effects and run the risk of losing their effectiveness over time due to acquired cancer cell resistance mechanisms.[26] A constant interaction between HSP and the adenosine triphosphate-binding (ATP)-binding region of the HER2 tyrosine kinase domain has been shown by *in silico* research. It has been demonstrated that tyrosine kinase signaling is the mechanism by which HSP induces cellular death and cell cycle stoppage in HER2-positive cell lines like SKBR3.[27] Therefore, HSP is a promising targeted anticancer treatment that may be able to block HER2 kinase activity or downregulate HER2 expression with little to no adverse issues.[28]

The DNA mismatch repair (MMR) system in cells protects against gene mutation and maintains genetic stability. The MMR system relies on two core dimers, MutL (MLH1 and/or PMS2) and MutS (MSH2 and/or MSH6), to recognize and rectify DNA mismatches.[29] MSH2 and MLH1 must remain intact in order to maintain PMS2 and MSH6 stability, respectively. However, even in the absence of MSH6 or PMS2, the stability of MLH1 and MSH2 can be maintained due to compensation by other MMR proteins. Through the use of immunohistochemical analysis of particular proteins, the severity of mismatch repair failure can be calculated.[30] MSH2 deletion or mutation may lead to genomic instability and tumors have shown increased rates of response treatment regimens involving oxaliplatin in patients with advanced non-small cell lung cancer. In response to oxidative stress, the p38 MAPK and c-Jun N-terminal kinase pathways encourage the ectopic synthesis of MSH2.[31,32] Tumor cell lines that have been specifically chosen for resistance to methylating medicines, cisplatin, and doxorubicin exhibit a loss of the MMR protein MLH1. Malignancies and cisplatin-resistant cell lines have hypermethylation, which reduces MLH1 expression.[33] When MMR function is restored by chromosomal transfer, MLH1-deficient tumor lines become more susceptible to a number of therapeutically significant drugs.[28,34]

Therefore, the study's goal was to find out how HSP affected various breast cancer cell lines, especially HER2-positive cell lines, which are believed to be the most aggressive form of the disease.

MATERIALS AND METHODS

SKBR3 and MCF-10a cell lines were gifted by Pasteur Institute, Baghdad, Iraq. Dulbecco's Modified Eagle's Medium (DMEM), penicillin/streptomycin (P/S), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) powder, and fetal bovine serum (FBS) were purchased from Gibco, USA. Real-time polymerase chain reaction (RT-PCR) strip was purchased from Gunster Biotech, Taiwan.

Cell culture

For cell culture, two distinct breast carcinoma cell lines, SKBR3 and MCF-10a, were employed. The cells were sustained in DMEM, with 10% FBS and 1% antibiotic supplement (P/S) to maintain their viability. Thereafter, the cell culture media was withdrawn and 5 mL of new culture medium was added. The cells were subjected to incubation that was adjusted at 37°C and 5% CO2. Throughout the week, the density of the culture flasks was monitored, and cell passage was done as required.[35]

Afterward to calculate the cell passage of SKBR3 and MCF-10a cell lines, the following formula was applied:

Cell density (cells/mL) = Total number of cells/Volume of medium used for counting (mL)

Cell backup and cryopreservation

The cells were prepared for cryopreservation by administering 1 mL of trypsin to separate them from the culture flask's bottom and discarding the supernatant. The supernatant was eliminated after the cells were rinsed with 5 mL of phosphate-buffered saline. In 1 mL of DMEM culture media, the cell plate was reconstituted. A Neobar slide is used to add 2 cells to the cryovial in accordance with the suggested procedure, which also calls for 90% FBS, 10% DMSO, and 2 × 106 cancer cells. The temperatures for cryovials were −20°C for 2 h, −80°C for 24 h, and −196°C during transfer to nitrogen tanks.[36]

Cell culture and treatment

To study how HSP affects each cancer cell line, the two cancer cell lines were kept in DMEM media with 10% FBS and 1% P/S. Trypan blue dye hemocytometers were used to collect and count the cells once they have reached the desired condition. The cells were then given various treatments and incubated for the desired amount of time at 37°C with 5% CO2. Cells from passage 3 were utilized in all studies, and 2% FBS was given to the cells to mimic starving.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell viability test

A 96-well plate with 10^4 cells was seeded with 200 L of DMEM media with 10% FBS and various doses of HSP (100, 200, 400, 600, 800, and 1000 µg/mL) for 48 h at 37°C with 5% CO2 to perform the MTT test. After 3 h of incubation, the surface culture media was removed from each well and 150 L of MTT solution (0.5 µg/mL) was added. The formazan precipitate was then dissolved using 100 L of DMSO after the MTT solution was withdrawn.[37] A spectrophotometer was used to measure the solution's absorbance at 570 and 630 nm. In order to determine cell viability, the following formula was utilized:

Cell viability = (Intensity of control absorption/Intensity of sample absorption) × 100.

Real-time polymerase chain reaction technique

It is an extremely sensitive method for determining mRNA in small samples. The amplification of the PCR products throughout each cycle allows for quantification. The PCR reaction is composed of three stages: exponential growth, plateau, and initiation. In this study, cyber green fluorescent dye was chosen since it is inexpensive and widely available. When it binds to any double-stranded DNA, including contaminants, primer dimers, and other nonspecific reaction byproducts, cyber green yields an estimate of the target concentration. The melting curve may be used to detect impurities, primer dimers, and nonspecific DNA. The melting temperature is the same for every PCR product generated by a certain pair of primers. The device gradually raises the temperature between 65°C and 95°C while observing the fluorescence at each location in order to create a melting curve. Using RT-PCR, the expression of the MSH2 gene in SKBR3 cells was assessed throughout the current experiment at varied HSP doses.[38]

The flow cytometric process

To find out how HSP lowers cell viability, flow cytometry was used to perform an apoptosis test and a cell cycle experiment. This technique distinguishes between early, late, and dead cells. The effect of varying concentrations of HSP (200, 400, and 600 µg/mL) on apoptosis was investigated using flow cytometric analysis. The SKBR3 and MCF-10A cells were labeled with annexin V and propidium iodide (PI) reagent. After a designated treatment duration, the SKBR3 and MCF 10A cells were harvested, washed, and subjected to staining with annexin V and PI reagent. Following the incubation, the stained cells were diluted in a buffer solution. A flow cytometer equipped with appropriate lasers and detectors for detecting fluorescence emitted by annexin V and PI was used. The acquired data from each stained cell suspension was processed and analyzed using the software. Gating strategies were employed to distinguish and classify distinct cell populations.[39,40]

FINDINGS AND ANALYSIS

Hesperetin's cytotoxic effect on SKBR3 cancer cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

HSP's impact on the cytotoxic response of MCF10-A and SKBR3 cell lines was assessed. After 48 h of exposure, the cytotoxic impact of HSP was assessed using the MTT test. Results indicate that there was no significant cytotoxic effect of HSP against SKBR3 cells at concentrations of 100, 200, and 400 µg/mL. However, a dose-dependent reduction in SKBR3 cell viability was observed at concentrations of 600, 800, and 1000 µM/mL, with a maximum inhibition rate of 82.55% ±3.01% of SKBR3 cells observed at 1000 µg/mL. Figure 1 provides a summary of the results.

MCF-10A cells were shown to be less responsive to the effects of HSP therapy than SKBR3 cells. At doses of 200 and 400 µM/mL, the pattern of cell inhibition did not change significantly. HSP did, however, significantly (P = 0.05) reduced cell viability at doses of 600, 800, and 1000 µM/mL, with a maximal inhibition rate of 48.278.73% seen at 1000 g/mL. At a dosage of 800 and 1000 µM/mL, the current research exhibited significant differences (P = 0.0008 and P = 0.0001, respectively) in the pattern of cell inhibition between SKBR3 and MCF-10A

cells, with SKBR3 cells displaying more sensitivity to HSP treatment than MCF-10A cells. HSP's half-maximal inhibitory concentration values for SKBR3 and MCF-10A cells were determined to be 289.6 and 855.4 μM/mL, respectively, as shown in Figure 2.

Hesperetin's cytotoxic effects on the expression of the SKBR3, MLH1, and MSH2 genes

The current work used RT-PCR to examine the impact of different HSP dosages (200, 400, and 600 g/mL) on the MLH1 and MSH2 gene expression in the SKBR3 breast cancer cell line, as shown in Figure 3. As higher concentrations of HSP were found to increase the expression of MLH1 and MSH2 genes in SKBR3 cells, the results showed that HSP concentration had an inverse relationship with the activity of cancer cells. Particularly, MLH1 gene expression changed in response to varying HSP concentrations.

The median MLH1 gene expression in the control group was 0.9669, whereas it increased to 1.938, 3.479, and 7.613 in the HSP200, HSP400, and HSP600 groups, respectively. The 25th percentile and 75th percentile of MLH1 gene expression also showed a similar increasing trend with increasing HSP concentrations. The results of the data's statistical analysis revealed a difference in MLH1 gene expression between the control group and the HSP400 and HSP600 groups that was statistically significant ($P = 0.05$). However, there was no discernible variance in the regulation of the MLH1 gene among the control group and the HSP200 group ($P > 0.05$).

In conclusion, the study suggests that higher concentrations of HSP may increase MLH1 gene expression in SKBR3 cells, indicating a potential therapeutic effect of HSP against breast cancer.

For the expression of the MSH2 gene in SKBR3 cells, results demonstrated that in the HSP200, HSP400, and HSP600 groups, the median MSH2 gene expression rose from 0.8488 in the control group to 2.289, 7.307, and 8.481 in those groups, respectively, as shown in Figure 4. HSP concentrations rose along with the 25th percentile of MSH2 gene expression, which went from 0.3455 in the control group to 6.473 in the HSP600 group. Similar to this, the 75th percentile of MSH2 gene expression increased in the HSP600 group from 10.34 to 1.433 in the control group.

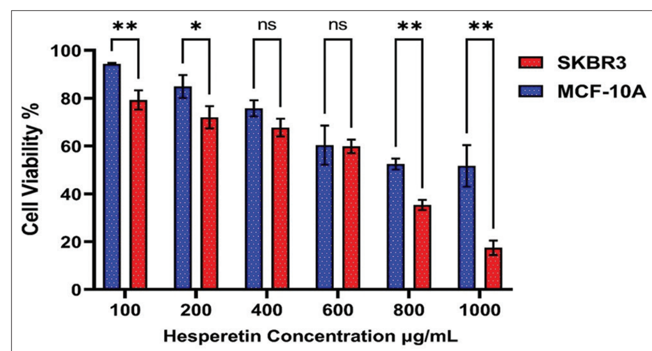

Figure 1: Mean (%) ± standard deviation of SKRB3 and MCF-10A cell viability after treatment with increasing concentrations of hesperetin for 24 h at 37°C. $^{**}P < 0.01$, $^{*}P < 0.05$. NS: nonsignificant

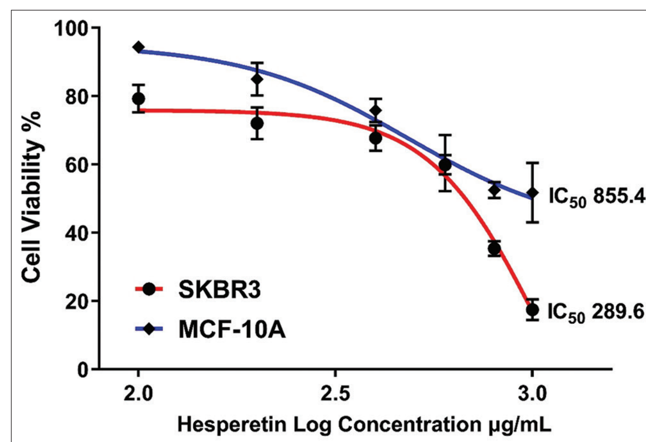

Figure 2: Dose–response curve (half-maximal inhibitory concentration) for SKRB3 and MCF-10A cells treated with hesperetin after 24 h incubation at 37°C

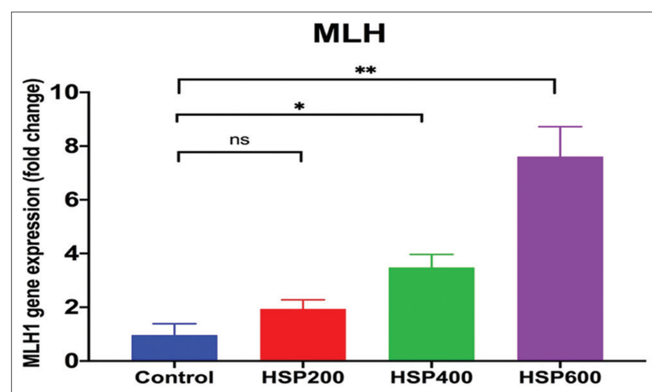

Figure 3: MLH1 gene expression of SKBR3 according to different HSP concentrations. HSP: Hesperetin, NS: nonsignificant

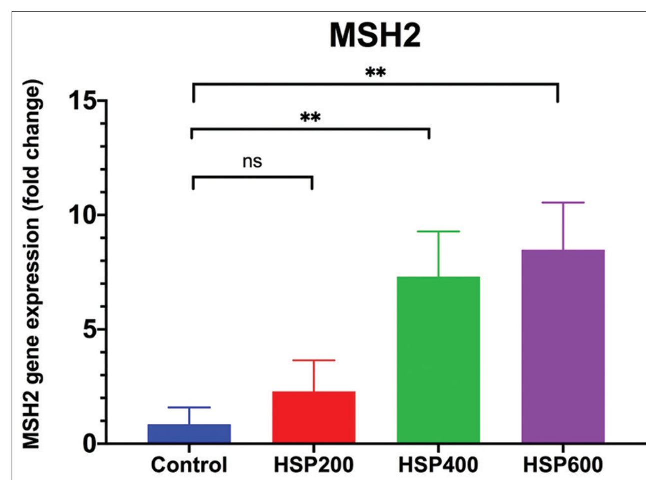

Figure 4: MSH2 gene expression of SKBR3 according to different HSP concentrations. HSP: Hesperetin, NS: nonsignificant

With $P < 0.001$, statistical analysis showed a very significant difference in MSH2 gene expression between the control group and the HSP400 and HSP600 groups. With $P = 0.833$, which is higher than the significance threshold of 0.05, there was no discernible change in MSH2 gene expression between the control group and the HSP200 group. The data suggest that higher concentrations of HSP may increase MSH2 gene expression in SKBR3 cells.

The MCF-10A breast normal cell line's MLH1 and MSH2 gene expression did not significantly alter in response to various HSP doses, according to the research. There were no clear trends or significant changes between the control and HSP-treated groups in the median gene expression levels or the 25th and 75th percentiles. Any detected variations in gene expression may have been the result of chance, according to statistical analysis using the nonparametric Kruskal–Wallis test, since the *P* values for all three comparisons were higher than the standard significance threshold of 0.05.

Measurement cytotoxic effect of hesperetin against SKBR3 cancer cell line used flow cytometric analysis

The results of the flow cytometry [Figure 5] exhibited that treatment with HSP at a concentration of 200 μg/mL elevated the population of apoptotic and dead cells to 19.3% and 15.9%, respectively, compared to untreated cells. Further increases in HSP concentration to 400 and 600 μg/mL markedly elevated the population of dead cells to 30.1% and 38.3%, respectively, with a maximum reduction in live cells to 43.4% at 600 μg/mL. The higher concentrations of HSP induced the killing of SKBR3 cells, as indicated by the MTT experiment.

CONCLUSIONS

HSP holds potential as a breast carcinoma therapy since it has been discovered to inhibit the growth of SKBR3 breast carcinoma cells in a concentration-dependent way. In addition, HSP can cause these cancer cells to undergo

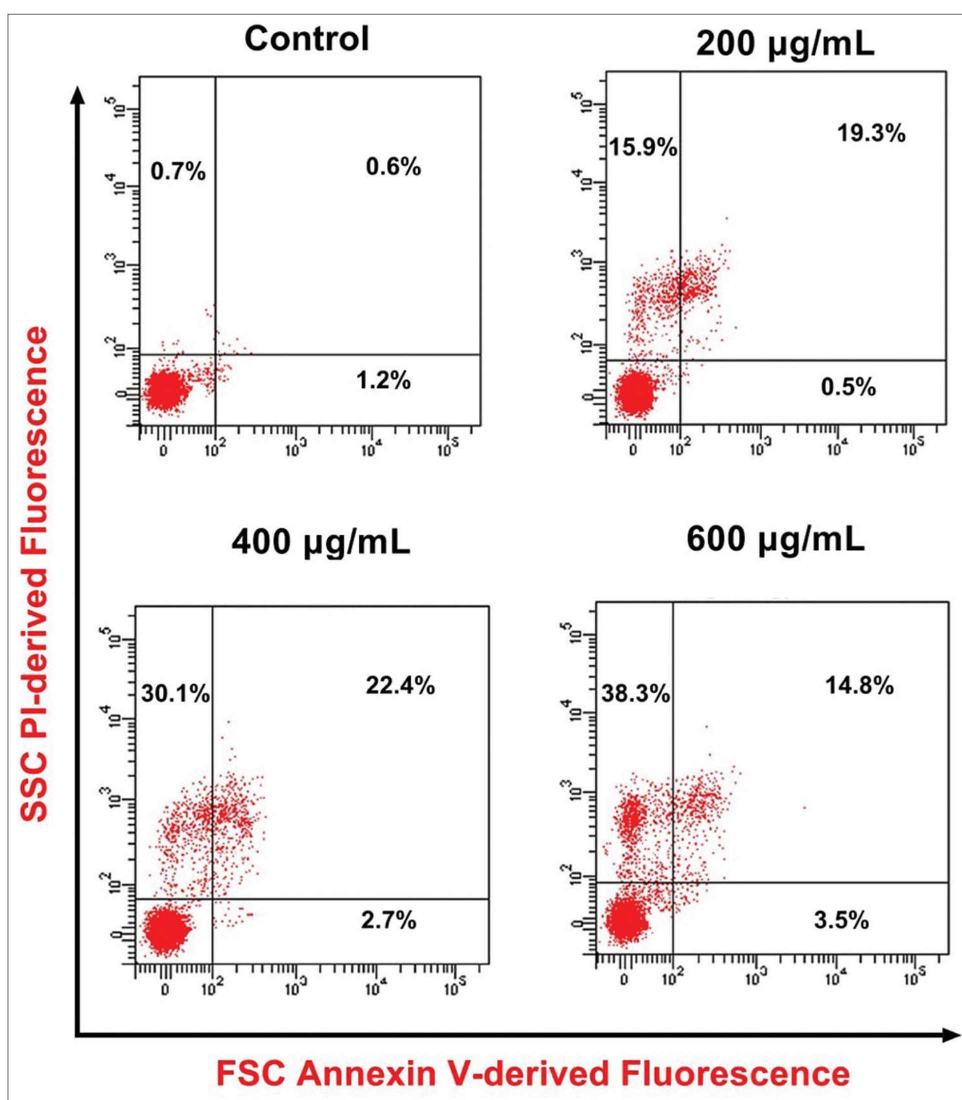

Figure 5: The apoptotic cell activity of hesperetin on breast cancer cell line (SKBR3) measured by flow cytometry. SSC: Side scatter, FSC: Forward scatter

concentration-dependent apoptosis, which results in cell death. High HSP concentrations may reduce the viability of SKBR3 cells while increasing their cytotoxicity. High amounts of HSP have been discovered to boost the expression of MLH1 and MSH2 genes, which are important in repairing DNA mutations and mismatch sequences. This suggests that HSP may also help these cancer cells' DNA repair processes. In addition, it has been discovered that HSP inhibits metastasis by stopping cancer cells' cell cycles, slowing down cell migration, and downregulating the expression of HER2, Rac1, and MMP9. These results imply that HSP could someday be investigated as a co-chemotherapeutic medication.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, *et al.* Molecular portraits of human breast tumours. Nature 2000;406:747-52.
2. Sonnenschein C, Soto AM. Carcinogenesis explained within the context of a theory of organisms. Prog Biophys Mol Biol 2016;122:70-6.
3. Maffini MV, Soto AM, Calabro JM, Ucci AA, Sonnenschein C. The stroma as a crucial target in rat mammary gland carcinogenesis. J Cell Sci 2004;117:1495-502.
4. Polyak K. Breast cancer: Origins and evolution. J Clin Invest 2007;117:3155-63.
5. Basse C, Arock M. The increasing roles of epigenetics in breast cancer: Implications for pathogenicity, biomarkers, prevention and treatment. Int J Cancer 2015;137:2785-94.
6. Al Khafaji S, Hade IM, Al Naqqash MA, Alnefaie GO. Potential effects of miR146 expression in relation to malondialdehyde as a biomarker for oxidative damage in patients with breast cancer. World Acad Sci J 2023;5:1-9.
7. Al-Khafaji AS, Pantazi P, Acha-Sagredo A, Schache A, Risk JM, Shaw RJ, *et al.* Overexpression of HURP mRNA in head and neck carcinoma and association with *in vitro* response to vinorelbine. Oncol Lett 2020;19:2502-7.
8. Al-Khafaji AS, Davies MP, Risk JM, Marcus MW, Koffa M, Gosney JR, *et al.* Aurora B expression modulates paclitaxel response in non-small cell lung cancer. Br J Cancer 2017;116:592-9.
9. Al-Khafaji AS, Marcus MW, Davies MP, Risk JM, Shaw RJ, Field JK, *et al.* AURKA mRNA expression is an independent predictor of poor prognosis in patients with non-small cell lung cancer. Oncol Lett 2017;13:4463-8.
10. Valenti G, Quinn HM, Heynen GJ, Lan L, Holland JD, Vogel R, *et al.* Cancer stem cells regulate cancer-associated fibroblasts via activation of hedgehog signaling in mammary gland tumors. Cancer Res 2017;77:2134-47.
11. Tan-Wong SM, French JD, Proudfoot NJ, Brown MA. Dynamic interactions between the promoter and terminator regions of the mammalian BRCA1 gene. Proc Natl Acad Sci U S A 2008;105:5160-5.
12. Bane AL, Beck JC, Bleiweiss I, Buys SS, Catalano E, Daly MB, *et al.* BRCA2 mutation-associated breast cancers exhibit a distinguishing phenotype based on morphology and molecular profiles from tissue microarrays. Am J Surg Pathol 2007;31:121-8.
13. Ménard S, Pupa SM, Campiglio M, Tagliabue E. Biologic and therapeutic role of HER2 in cancer. Oncogene 2003;22:6570-8.
14. Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release 2010;146:264-75.
15. Davis NM, Sokolosky M, Stadelman K, Abrams SL, Libra M, Candido S, *et al.* Deregulation of the EGFR/PI3K/PTEN/Akt/mTORC1 pathway in breast cancer: Possibilities for therapeutic intervention. Oncotarget 2014;5:4603-50.
16. Maughan KL, Lutterbie MA, Ham PS. Treatment of breast cancer. Am Fam Physician 2010;81:1339-46.
17. Hance KW, Anderson WF, Devesa SS, Young HA, Levine PH. Trends in inflammatory breast carcinoma incidence and survival: The surveillance, epidemiology, and end results program at the national cancer institute. J Natl Cancer Inst 2005;97:966-75.
18. Earl HM, Hiller L, Vallier AL, Loi S, McAdam K, Hughes-Davies L, *et al.* 6 versus 12 months of adjuvant trastuzumab for HER2-positive early breast cancer (PERSEPHONE): 4-year disease-free survival results of a randomised phase 3 non-inferiority trial. Lancet 2019;393:2599-612.
19. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: Patient-level meta-analysis of the randomised trials. Lancet 2015;386:1341-52.
20. Pagani O, Regan MM, Walley BA, Fleming GF, Colleoni M, Láng I, *et al.* Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. N Engl J Med 2014;371:107-18.
21. Asselain B, Barlow W, Bartlett J, Bergh J, Bergsten-Nordström E, *et al.* Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: Meta-analysis of individual patient data from ten randomised trials. Lancet Oncol 2018;19:27-39.
22. Pivot X, Romieu G, Debled M, Pierga JY, Kerbrat P, Bachelot T, *et al.* 6 months versus 12 months of adjuvant trastuzumab for patients with HER2-positive early breast cancer (PHARE): A randomised phase 3 trial. Lancet Oncol 2013;14:741-8.
23. Chandrika BB, Steephan M, Kumar TR, Sabu A, Haridas M. Hesperetin and naringenin sensitize HER2 positive cancer cells to death by serving as HER2 tyrosine kinase inhibitors. Life Sci 2016;160:47-56.
24. Yap KM, Sekar M, Wu YS, Gan SH, Rani NN, Seow LJ, *et al.* Hesperidin and its aglycone hesperetin in breast cancer therapy: A review of recent developments and future prospects. Saudi J Biol Sci 2021;28:6730-47.
25. Bak Y, Kim H, Kang JW, Lee DH, Kim MS, Park YS, *et al.* A synthetic naringenin derivative, 5-hydroxy-7,4'-diacetyloxyflavanone-N-phenyl hydrazone (N101-43), induces apoptosis through up-regulation of Fas/FasL expression and inhibition of PI3K/Akt signaling pathways in non-small-cell lung cancer cells. J Agric Food Chem 2011;59:10286-97.
26. Gajria D, Chandarlapaty S. HER2-amplified breast cancer: Mechanisms of trastuzumab resistance and novel targeted

therapies. Expert Rev Anticancer Ther 2011;11:263-75.

27. Brunner K, Fischer CA, Driemel O, Hartmann A, Brockhoff G, Schwarz S. EGFR (HER) family protein expression and cytogenetics in 219 squamous cell carcinomas of the upper respiratory tract: ERBB2 overexpression independent prediction of poor prognosis. Anal Quant Cytol Histol 2010;32:78-89.
28. Malik SS, Masood N, Asif M, Ahmed P, Shah ZU, Khan JS. Expressional analysis of MLH1 and MSH2 in breast cancer. Curr Probl Cancer 2019;43:97-105.
29. Westenend PJ, Schütte R, Hoogmans MM, Wagner A, Dinjens WN. Breast cancer in an MSH2 gene mutation carrier. Hum Pathol 2005;36:1322-6.
30. Caluseriu O, Cordisco EL, Viel A, Majore S, Nascimbeni R, Pucciarelli S, *et al*. Four novel MSH2 and MLH1 frameshift mutations and occurrence of a breast cancer phenocopy in hereditary nonpolyposis colorectal cancer. Hum Mutat 2001;17:521.
31. Bianchi F, Raponi M, Piva F, Viel A, Bearzi I, Galizia E, *et al.* An intronic mutation in MLH1 associated with familial colon and breast cancer. Fam Cancer 2011;10:27-35.
32. Lanza G, Gafà R, Santini A, Maestri I, Guerzoni L, Cavazzini L. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. J Clin Oncol 2006;24:2359-67.
33. Ko JC, Peng YS, Wu CH, Chen JC, Zheng HY, Lin YC, *et al*. Down-regulation of MutS homolog 2 (MSH2) expression by curcumin enhances cytotoxicity induced by gemcitabine in human lung adenocarcinoma cells. Epidemiol 2017;7:332.
34. Lopez D, Sekharam M, Coppola D, Carter WB. Purified human chorionic gonadotropin induces apoptosis in breast cancer. Mol Cancer Ther 2008;7:2837-44.
35. Green AR, Aleskandarany MA, Agarwal D, Elsheikh S, Nolan CC, Diez-Rodriguez M, *et al.* Myc functions are specific in biological subtypes of breast cancer and confers resistance to endocrine therapy in luminal tumours. Br J Cancer 2016;114:917-28.
36. Jung M, Russell AJ, Liu B, George J, Liu PY, Liu T, *et al*. A Myc activity signature predicts poor clinical outcomes in Myc-associated cancers. Cancer Res 2017;77:971-81.
37. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: Weaving a tumorigenic web. Nat Rev Cancer 2011;11:761-74.
38. Fernández-Medarde A, Santos E. Ras in cancer and developmental diseases. Genes Cancer 2011;2:344-58.
39. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7-30.
40. Vembadi A, Menachery A, Qasaimeh MA. Cell cytometry: Review and perspective on biotechnological advances. Front Bioeng Biotechnol 2019;7:147.