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DETERMINATION OF THE EFFECTS OF IRISIN HORMONE IN SKMEL-30 CELLS

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Melanocytes, the skin's pigment-producing cells, are the source of the skin cancer known as melanoma. Numerous variables, including as immune system interactions, tumor microenvironment, and genetic alterations, have an impact on the development and behavior of melanoma. The purpose of this study was to ascertain the impact of irisin on melanoma cells. The molecular effects of irisin SKMEL-30 on human melanoma cancer cells were examined for this aim. By using MTT technique, the effects of irisin on cell growth were examined. Real-time polymerase chain reaction was used to examine changes in gene expression level. The concentrations of sialic acid were measured using spectrophotometry. In the investigation, the irisin IC50 value for a 24-hour application was determined to be 30 nM. In comparison to the control group, sialic acid levels in the irisin-treated group of SKMEL-30 cells were significantly lower. In the qRT-PCR investigation, ST8SIA-2, one of the glycosyltransferase genes, increased 12.591-fold in the application group whereas cas8, one of the apoptotic genes, increased 82.481-fold. In conclusion, flow cytometry analyses proved that administration of 30 nM irisin to SKMEL-30 cells influences cell proliferation but does not cause apoptosis. It was shown that sialic acid substitution reduced the proliferative and metastatic potential of SKMEL-30 cells.

Keywords: SKMEL-30, Melanoma, Sialic Acid, Sialiltransferase, MTT

INTRODUCTION

Melanocytes, which are pigment-producing skin cells, are the site of melanoma cancer. The skin, eyes, and mucous membranes all include melanocytes, which are the cells that make the pigment melanin. Melanoma can grow in other parts of the body, although it often appears in moles

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and other pigmented regions of the skin. In general, melanoma development may be influenced by variables including skin type, genetic susceptibility, and exposure to sunlight or ultraviolet (UV) radiation. Because it may spread (metastasize) swiftly and affect other organs, melanoma is a more dangerous kind of cancer than other skin cancers (RASTELLI *et al*., 2014; AHMET *et al*., 2020). Dermatologists will examine your skin and do a biopsy of the lesion before determining whether you have melanoma. The stage of the melanoma, how far it has progressed, and the patient's overall condition all influence the available treatments (DZWIERZYNSKI, 2021). Surgery, immunotherapy, targeted treatment, and radiation are among the techniques employed. In situations of early-stage melanoma, surgery is typically the backbone of therapy; however, combined treatments are employed in cases of advanced-stage melanoma (DZWIERZYNSKI, 2021; BOBOS, 2021).

Signaling pathways are intricate biological networks that allow for cell-to-cell contact and signal transmission. Cancer development, growth, metastasis, cell survival, apoptosis (programmed cell death), and angiogenesis (vascular creation) are all controlled by signaling pathways in cancer cells (PARK *et al*., 2020; ULLAH *et al.,* 2022). Through the use of the proper signaling pathways, normal cells maintain a balance between cell survival and apoptosis. Cancer cells, however, have a high capacity for survival due to the deactivation of their apoptotic systems. Tumors develop as a result of the buildup of cancerous cells as extracellular matrix. In cancer cells, pro-survival pathways including PI3K/AKT signaling and anti-apoptotic molecules like the Bcl-2 family of proteins are typically overexpressed (SORICE, 2022).

Metastasis is the spread of cancer cells from the original tumor to other parts of the body. Signaling pathways play an important role in the metastasis process. For example, Wnt/β-catenin pathways regulate the expression of genes that affect cell migration and invasion. Metastatic cancer cells can acquire invasive properties by overactivation or altered regulation of signaling pathways (SUHAIL *et al*., 2019). Regulation of signal pathways in cancer cells occurs with factors such as gene mutations, changes in gene regulatory factors or excessive or incorrect activation of signal pathway components. These irregularities can increase the abnormal growth, division, survival, and metastasis abilities of cancer cells. Therefore, many of the cancer treatment strategies focus on drugs and therapeutic agents that target signaling pathways (SUHAIL *et al*., 2019; FARES *et al.,* 2020).

A sugar acid known sialic acid is a part of the glycoproteins and glycolipids that are located on the surface of cells. Sialic acid is crucial for the identification of cell-surface receptors, cell signaling, and interactions with the matrix. Many cancer forms, including melanoma cancer, have sialic acid regulatory alterations that have been seen to affect the activation of signaling pathways and the development of cancer. By binding to cell-surface receptors and signal transduction molecules or by affecting the glycosylation state of these components, sialic acid affects signaling pathways. These interactions influence how signaling pathways that control a number of biological activities, including cell signaling, cell growth, division, migration, invasion, metastasis, and death, are activated or inhibited. Melanoma cell's ability to metastasize is increased by sialic acid. Increased sialic acid expression can alter the metastatic pathways and improve the invasiveness of melanoma cells (LAUBLI *et al*., 2022). Through the control of cell-matrix interactions and the influence of certain cell surface receptors, sialic acid has been found to facilitate the migration and invasion of melanoma cells. Sialic acid expression may be increased to aid melanoma cells in suppressing the immunological response. It is well known that sialic acid can influence how

immune cells are recognized and activated. This may lead to melanoma cells evading the immune system and preventing the immune system from detecting the tumor. Increases in anti-apoptotic factors and intracellular signaling pathways may result from increased sialic acid expression. Melanoma cells may be more likely to last longer and acquire resistance as a result. The use of sialic acid as a predictive marker in melanoma cancer has been studied. Sialic acid expression may be elevated as a sign of illness progression and a bad prognosis. Additionally, one prospective technique for the treatment of melanoma is the creation of sialic acid-targeted medicines (CASSETTA *et al.,* 2019).

Most literature research is needed in the area of sialic acid's function in melanoma cancer. Knowing how sialic acid affects the signaling pathways used by melanoma cancer may help us better understand how the disease develops and how to treat it. More studies are needed to fully understand the processes by which sialic acid affects the activation or inhibition of signaling pathways. However, it appears that sialic acid may alter the activation or inhibition of signaling pathways by interacting with cell surface receptors and signaling molecules. The purpose of choosing irisin hormone in this study is; Although this hormone does not seem to activate NF-κB in myocytes, it inhibits p38 mitogen-activated protein kinase (p38 MAPK), causing apoptosis by reducing the expression of inflammatory genes. In addition, irisin also reduces oxidative stress in macrophages and endothelial cells. Therefore, our study aimed to examine the effect of irisin hormone on signaling pathways and sialic acid change in melanoma cancer SKMEL-30 cells. In this context, IC50 dose was determined by performing MTT test on SKMEL-30 cells exposed to irisin hormone. After dose determination studies, qRT-PCR analysis was performed to examine the changes caused by the LD_{50} dose in the cell, and apoptotic, metastatic, oncogenic genes, as well as glycosyltransferase genes, were investigated. In addition, sialic acid residues in SKMEL-30 cells treated with irisin hormone were compared to the control group, and its effect on the metastatic cascade was evaluated.

MATERIALS AND METHODS

Culturing cells

Melanoma (SKMEL-30) cell line was used in the study. The cell line was grown in the Safe Fast Elite (EN 12469 2000) flow chamber. For cell passage and culture, DMEM, EMEM-HamsF-12 (MULTICELL), 1% L-glutamine (MULTICELL (609-065-E2), 1% penicillin-streptomycin (MULTICELL (450-201-Z2) Bovine serum-containing medium (MULTICELL (FBS-HI-IIA)) was utilized, and it was incubated at 37 °C and 5% CO2 in sterile incubators (PANASONIC). Cultured cell lines were repeated up to the fifth passage, at which point stocks were made ready for use. DMSO (MERCK 67-68-15) was placed in medium-containing cryotubes, frozen in liquid nitrogen, and kept at a temperature of -150°C (PANASONIC). Every research project began at the fifth passage of the cell lines and ended at the fifteenth passage.

Analysis of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)

Cell viability and proliferation were evaluated using the MTT technique. In a 96-well plate consisting of SKMEL-30 cells, 2000 cells/well were seeded on cell culture plates. To support cell adhesion, it was incubated in DMEM:EMEM:Ham'sF12 medium for 24 hours. By scanning the literature, the dose range to be administered of the irisin hormone was determined. Irisin hormone was applied in the range of 3.75-120 nanoMolar (nM) as a serial dilution in ultrapure

water. Eight replicates for each dose were run in treated cells over a 24-hour incubation period. Following this, 20 microliters of MTT solution (5 mg/ml concentration) was administered to each well and a two-hour incubation period was applied to each well at 37°C. Blue formazan was dissolved in ultrapure dimethyl sulfoxide (100–200 µl/well) and a microplate reader (Multiscan Go, Thermo Scientific, USA) was used to evaluate the results at 492 nm. Probit analysis using SPSS 20 software was used to obtain LD50 values.

Fluorescent Microscopy Analysis

SKMEL-30 cells were seeded in 6-well plates (NEST) at $5x10^4$ cells per well and incubated for 24 hours at 37°C in 5% CO_2 incubator. 30 nM İrisin was applied to examine its molecular effects. After 24 hours, a batch was stained with fluorescent dyes Annexin V (Elabscience, USA) and Hoechst 34580 (Santa Cruz, USA).

For Annexin V staining, each well received 200 l of Annexin V-FITC diluted in 1.8 ml of 1 m Annexin V binding buffer (1:10) for 20 minutes at room temperature and in the dark. Each well's whole contents were drained after 20 minutes, and the cells were then given a single wash with 1x PBS. Under a fluorescent (Zeiss Observer Z1, Germany) microscope, cell pictures were obtained on a 20-objective using DAPI and FITC channels in order to prevent the cells from drying out. 1 PBS was added to cover the cells to keep them from drying out. To demonstrate cell division and apoptotic processes at cellular scale under a fluorescent microscope, 25 g of Hoechst 34580 dye was dissolved in 50 mL of 1 PBS. After removing the media covering the cells, 2 mL of Hoechst 34580 solution was poured into each well and left there for 20 minutes in the dark and at room temperature. The cells were seen using a fluorescence microscope with a 20 objective at the conclusion of the application time using the DAPI channel.

Determination the Levels of Sialic Acid

SKMEL-30 cells were planted on 6-plate plates, and the cells were then incubated for 24 hours at 37°C with 5% CO2. 30 nM irisin hormone was administered and incubated for 24 hours at the conclusion of the period. In 0.1 M phosphate buffer (pH 7.4), control and SKMEL-30 cells that had been treated with 30 nM irisin were homogenized for 5 minutes. Homogenized samples in the amount of 0.2 ml were obtained, made up to 1 ml with a solution of 5% perchloric acid, and then incubated at 100°C for 5 minutes. Using phosphate buffer that was made between 0.1 M and pH 7.4, a solution of 2.5 mg/ml sialic acid (N-acetyl neuraminic acid) was created. Standard procedures were devised, and the prepared stock solution was diluted. Ehrlich's reagent was added to the calibration procedures and incubated with the samples for four hours at 100°C. After taking spectrophotometric readings at 525 nm, graphs were created, and the amounts of sialic acid in the samples were estimated as mg/ml.

RNA isolation and cDNA synthesis

Total RNA was isolated from the SKMEL-30 cell line (Invitrogen TM-12183018A) using the Pure Link RNA isolation kit. NanoDrop (NanoQ Optizen) was used to measure the amount of RNA that was separated. cDNA Reverse Transcription Kit (Applied Biosystems-00709629) PCR conditions Step 1 was 25°C for 10 minutes; Step 2 was 37°C for 120 minutes; and Step 3 was 85°C for 5 minutes, during which cDNA synthesis was completed.

Real-Time PCR (qRT-PCR)

In this investigation, we employed a Quantstudio 6 Flex qRT-PCR system (Sybr Green Method (PowerSYBR Green-Applied Biosystems-1805575)) that reads 384-well microplates. The endogenous gene that was employed to examine the samples was GAPDH.

Statistical analysis

Cell amount calculations due to irisin application were made according to the formula "Cell Viability%: (absorbance value of the substance applied wells / absorbance value of the control wells) × 100". The LD_{50} doses were calculated using the SPSS Resgression Probit (IBM SPSS Statistics 22) application. Changes in gene expressions due to the application of 3 repetitive doses of IC50 were calculated with the formula $2^{-\Delta\Delta cT}$. Statistical difference between results was determined according to $p \leq 0.05$ value (SPSS-Anova (Duncan Test)). GAPDH mRNA was used as an internal control.

RESULTS

Within the parameters of the study, the MTT test—which is detailed in the Materials and Methods section—was used to assess the impact of irisin hormone on the viability of SKMEL-30 melanoma cell lines for a 24-hour period. The SKMEL-30 cell line experienced dose-related mortality on cell viability after being exposed to irisin hormone for 24 hours. The irisin hormone application resulted in an LD_{50} dosage of 30 nM. As seen in Figure 1, sialic acid amounts in SKMEL-30 cells treated with 30 nM irisin hormone were significantly reduced compared to the control group (mean±std.).

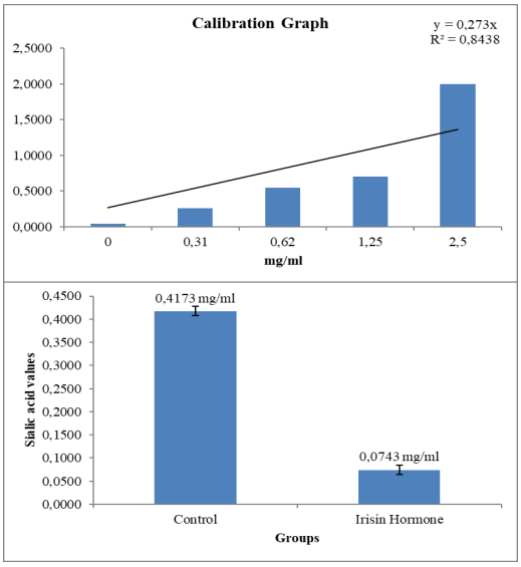

Figure 1. Spectrophotometric determination of sialic acid residues of control and irisin hormone-exposed SKMEL-30 cell line. All data were normalized to 5-point calibration and evaluated relative to control; Data are represented as mean±SD and given in mg/ml. Check: Vehicle treated,Irisin: 30 nM

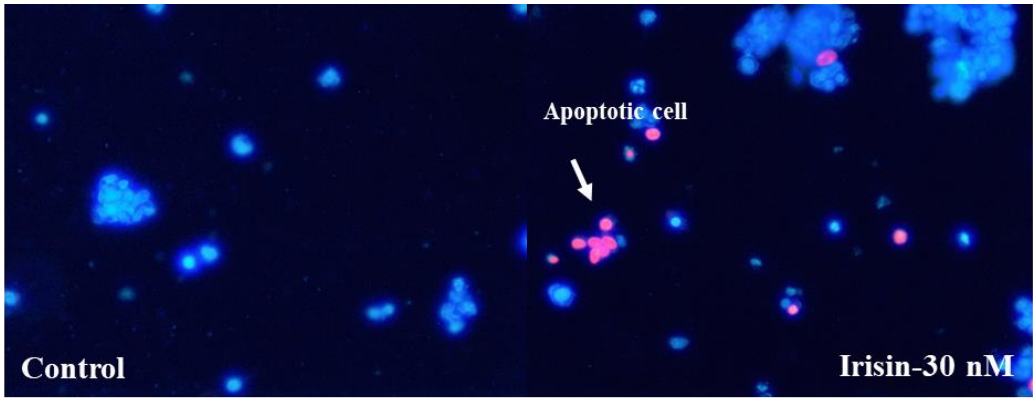

Figure 2. Detection of apoptotic cell fluorescence (CTCF) level of SKMEL-30 cells (Nuclear Blue: blue, Propidium Iodide: Red).

The reduction of sialic acid in cancer cells leads to various biochemical and cellular effects. Sialic acid is a carbohydrate component found in glycoproteins on the cell surface and regulates intercellular adhesion. Decreased sialic acid in cancer cells may result in impaired intercellular adhesion and reduced metastasis abilities. This limits the ability of cancer cells to spread and invade. As described in the material and technique section, cDNAs derived from RNAs recovered from melanoma cell lines were used in the study to detect changes in gene expressions by the qRT-PCR method. Three repetitions of the investigation were conducted, and statistical analyses were performed to assess the changes in gene expressions found in the SKMEL-30 Melanoma cells of the application group relative to the control group. As a result of the statistical analysis, in the irisin group compared to the control, the apoptosis genes Cas-3: 2.28 fold, Cas-8: 199.346 fold, Cas-9: 2.259 fold, Fas: 8.482 fold increased, while no statistical change was observed in the Cyc-c gene. These results made us think that the irisin hormone acts through the extrinsic pathway, not the intrinsic pathway. An increase was observed in metastasis genes: TIMP-1: 2,292-fold, TIMP-2: 2,440-fold, MMP-2: 3,095-fold, CDH-2: 3,488-fold. These increases showed that metastasis was suppressed. Among the glycosyltransferase genes; ST8Sia-2: increased by 21,682 fold, B3GNCT: increased by 3,647 fold, GALNAC-13: increased by 4,304 fold. It was thought that these increases were the result of decreasing sialic acid acid balances with the application of irisin hormone and that metastasis was suppressed due to these decreasing sialic acid balances on the cell surface. Irisin-induced changes in the expression of genes related to metastasis, glycosyltransferase, oncogene and apoptotic signaling pathway in SKMEL-30 Melanoma cell lines are presented in Table 1.

Table 1. Real time PCR gene Expression results. Relative fold change determined by quantitative real-time PCR (qRT-PCR) analysis of apoptosis, metastasis, oncegenic and glycosyltransferases genes in Control and irisin hormon exposed SKMEL-30 cell line. All data were normalized with GAPDH expression and given as relative to control; Data were represented mean±SD. Control: Vehicle treated, İrisin: 30 nM

Signal Pathways	Gene ID	Groups	Relative Quantification (n:3±std)
Apoptosis Genes	Caspase-3	Control	1,076±0,079
		Irisin	2,280±0,182
	Caspase-8	Control	31,221±1,164
		Irisin	199,346±6,324
	Caspase-9	Control	1,077±0,092
		Irisin	2,529±0,233
	Cyc-C	Control	1,268±0,069
		Irisin	1,795±0,302
	FAS	Control	0,797±0,157
		Irisin	8,482±0,393
Metastasis Genes	TIMP-1	Control	0,755±0,479
		Irisin	2,292±0,274
	TIMP-2	Control	1,198±0,157
		Irisin	2,440±0,327
	MMP-2	Control	1,410±0,133
		Irisin	3,095±0,248
	CDH-2	Control	1,385±0,166
		Irisin	3,488±0,333
Oncogenes	h-RAS	Control	2,656±1,066
		Irisin	4,088±0,422
	k-RAS	Control	0,539±0,206
		Irisin	1,435±0,285
	MEK	Control	1,846±0,844
		Irisin	2,475±0,171
	RAF	Control	1,341±0,230
		Irisin	2,691±0,376
	ERK	Control	1,214±0,250
		Irisin	2,234±0,629
Glycosyltransferase Genes	GNT-V	Control	1,300±0,343
		Irisin	1,809±0,175
	ST8Sia-2	Control	3,076±0,524
		Irisin	21,682±4,785
	B3GNCT	Control	1,834±0,664
		Irisin	3,647±0,625
	GALNAC-13	Control	1,512±0,644
		Irisin	4,304±0,515

DISCUSSION

One of the main causes of death for people is cancer. Many studies have shown increased irisin expression in cancer. However, several studies have also reported decreased irisin expression in cancer patients. Therefore, more research is needed to investigate the role of irisin in cancer. Irisin inhibits proliferation, migration, invasion, and epithelial-to-mesenchymal transition (EMT) in many cancer cells in vitro. Therefore, in our study, we investigated the mechanism of action of the irisin hormone in SKMEL-30 melanoma cells. A biomarker to differentiate oncocytic variations of thyroid cancer from other forms is irisin hormone immunoreactivity (UGUR *et al.*, 2019). Irisin was applied to obesity-associated cancer cell lines in 2014 at physiological (5 to 10 nmol/L) and higher physiological/pharmacological (50 to 100 nmol/L) concentrations by Moon and Mantzoros. Irisin was tested after 36 hours of incubation on the malignant potential and cell proliferation of obesity-associated cancer cell lines, including esophageal (OE13 and OE33), colon (HT29 and MCA38), thyroid (SW579 and BHP7), and endometrial (KLE and RL95-2). Its lack of in vitro effects has been established (MOON and MANTZORO, 2013). Irisin showed no influence on the malignant potential and cell proliferation of esophageal cancer cell lines (OE13 and OE33) in vitro, according to a 2016 study by AYDIN *et al*. (2016). Human lung cancer cells were treated by Shao et al. in 2016 with irisin at varying concentrations (0, 10, 20, and 50 nM) for varying lengths of time (0, 24, and 48 hours). The findings demonstrated that, in a time-dependent manner, irisin dramatically reduced lung cancer cell migration, invasion, and multiplication. E-cadherin expression rose in response to a rise in irisin concentration, whereas the expression of vimentin, N-cadherin, and snail decreased. Moreover, the action of irisin can be counteracted by the PI3K inhibitor LY294002. According to the research, irisin may be able to stop lung cancer cells from invasively utilizing the PI3K/Akt/Snail pathway and from going through the epithelial-mesenchymal transition (EMT) (SHAO *et al.*, 2017). Shi and associates discovered that irisin, through triggering the PI3K/AKT pathway and decreasing Dox's cytotoxicity, greatly enhanced cell invasion, migration, and proliferation (SHI *et al*., 2017). Liu *et al*., discovered that irisin, through blocking the PI3K/AKT/NF-kB signaling pathway, enhanced the chemosensitivity of pancreatic cancer cells to Dox or gemcitabine and doxorubicin-induced cell death in pancreatic cancer (LIU *et al.*, 2019).

Sialic acid regulates the adhesion between cells, so the reduction of sialic acid reduces the ability of melanoma cells to attach to each other and their ability to invade. This, in turn, contributes to the prevention of metastasis and the reduced ability of cancer cells to spread. Sialic acid is used by cancer cells as an immune escape mechanism. The reduction of sialic acid leads to better recognition and targeting of cancer cells by the immune system. This allows the immune system to detect and destroy cancer cells more effectively. In our study; qRT-PCR analysis using GAPDH as an endogenous control in SKMEL-30 Melanoma cell lines revealed the following, Caspase 8 showed a relative fold increase of 199.346±6,324 fold in the expression of irisin hormone apoptotic signaling pathway genes, Fas ligand (FasL) signaling molecule increased 8.482±0.393 fold, Caspase 8 activation triggered the apoptotic signaling pathway, increasing Caspase-3 gene expression 2.280±0.182 fold and caspase-9 gene expression 2.529±0.233 fold compared to the control. However, it was determined that these increases did not statistically change the activation of mitochondrial apoptosis genes.

ZHANG *et al*.'s (2019) study shown that irisin reduced the PI3K/AKT pathway's malignant development in human pancreatic cancer cells. Serum irisin has been shown to protect the spine from breast cancer metastases and to function as a novel marker for early detection and diagnosis of breast cancer in two distinct investigations (PROVATOPOULOU *et al.*, 2015; ZHANG *et al.*, 2018). FNDC5/Irisin was proposed by GAGGINI *et al*. (2018) as a possible therapeutic approach for the management of metabolic disorders and carcinogenesis (GAGGINI *et al.*, 2018). Irisin has been proposed as a possible blood diagnostic marker for colorectal cancer, according to a study (ZHU *et al.*, 2018). In 2017, KONG *et al*. applied irisin at varying concentrations (0, 25, 50, 100, and 200 ng/mL) to osteosarcoma cells U2OS and MG-63 at various time intervals (12, 24, and 48 h). The findings demonstrated that irisin reduced U2OS and MG-63 osteosarcoma cells' migration, invasion, and proliferation in a time- and dose-dependent manner. Meanwhile, via controlling the expression of matrix metalloproteinases (MMP)-2, MMP-7, MMP-9, vimentin, fibronectin, and E-cadherin, irisin reversed the EMT impact that IL-6 had generated in osteosarcoma cells. Furthermore, in osteosarcoma cells, irisin suppressed the expression of Snail triggered by IL-6 as well as the activation of STAT 3. Lastly, WP1066's suppression of STAT3 improved irisin's impact on EMT and Snail expression in osteosarcoma cells (KONG *et al.*, 2017).

Timp-1 gene increased 2.292±0.274 fold, TIMP-2 gene 2.440±0340 fold, MMP-2 gene 3.095±0.248 fold, CDH-2 gene 3.488±0.333 fold in SKMEL-30 cells to which 30 nM irisin hormone was applied. This increase in metastasis genes indicates that the inhibition of the activity of metalloproteinases decreases the destruction of the extracellular matrix and reduces the potential of SKMEL-30 cells to invade surrounding tissues and metastasize. The increase of Timp-1 may help cancer cells evade the immune response system. Timp-1 can prevent immune cells from detecting and destroying cancer cells. This can promote the growth and metastasis of cancer cells by escaping the immune system. The increase in the timp-1 gene may promote the aggressive properties and progression of cancer cells. Therefore, Timp-1 can be used as a prognostic or predictive marker in the evaluation of cancer diagnosis, prognosis and treatment strategies. TIMP-2 contributes to a process that normally regulates tissue destruction and remodelling. However, the increase in the TIMP-2 gene in cancer cells increases the invasion and metastasis abilities of cancer cells by destroying the metalloproteinases, extracellular matrix. The increase in the TIMP-2 gene decreases the degradation of the extracellular matrix as it inhibits the activity of metalloproteinases. The increase of TIMP-2 can reduce the metastatic potential of cancer cells. Metalloproteinases are factors that increase the invasion and metastasis abilities of cancer cells. TIMP-2 can limit the metastasis abilities of cancer cells by inhibiting the activity of metalloproteinases. CDH2 (Cadherin-2) is a gene encoded by N-cadherin, a transmembrane protein that provides adhesion between cells. CDH2 regulates cell-matrix interactions by providing adhesion between cells. The increase in the CDH2 gene can lead to overproduction of N-cadherin protein and altered cell-matrix interactions. This may increase the ability of cancer cells to attach to and invade the extracellular matrix. The increase in the DH2 gene may cause changes in intercellular adhesion. Normally, N-cadherin, which forms strong bonds between cells, can be overproduced with an increase in the CDH2 gene, and irregular bonds may occur between cells. This may increase the ability of cancer cells to attach tightly to each other and to migrate or metastasize. This increase in the CDH-2 gene indicates that SKMEL-30 cells trigger the activation of the Epithelial-Mesenchymal Transition (EMT) process. EMT expresses the transformation of

cells from epithelium to mesenchyme and plays an important role in cancer metastasis. It is thought that the application of irisin hormone activates the EMT process and contributes to the survival of cancer cells by suppressing apoptosis.

The H-RAS gene is known as an oncogene belonging to the RAS family. The increase in the H-RAS gene in cancer cells can affect important cellular processes such as cell growth, cell division, cell survival and cell cycle. H-RAS is GTPase enzyme that activates intracellular signalling pathways. The increase in the H-RAS gene may cause overproduction of the H-RAS protein and continuous activation of these signalling pathways. This can lead to increased cell growth and proliferation. Overactivation of H-RAS has been determined to activate cell survival signals and inhibit the apoptosis process. The increase in the H-RAS gene may promote the aggressive properties and progression of cancer cells. Therefore, therapies targeting the uncontrolled activation of the H-RAS gene may be a potential strategy in cancer treatment. The MEK (MAPK/ERK kinase) gene belongs to a family of genes that play an important role in cancer development. MEK is a loop of MAPK/ERK kinase pathways, a component of intracellular signal transduction pathways. These pathways regulate many cellular processes such as cell growth, cell proliferation, cell survival, cell migration and cell differentiation. Mutations or increased activation in the MEK gene can lead to sustained activation of the MAPK/ERK kinase pathways and increased cell growth and proliferation. This may contribute to the uncontrolled proliferation of cancer cells and tumor formation. Overactivation of the MEK gene can affect cell survival signals. Activated MEK enables the transmission of anti-apoptotic signals by activating the signalling pathways in the cell. This can lead to cancer cells preventing apoptosis and surviving uncontrollably. The RAF gene is a component of the RAS/RAF/MEK/ERK signalling pathways and plays an important role in regulating many cellular processes such as cell growth, cell proliferation, cell survival and cell differentiation. The increase in the AF gene may result in continuous activation of the RAS/RAF/MEK/ERK signalling pathways and increased cell growth and proliferation. This may contribute to the uncontrolled proliferation of cancer cells and tumor formation. Overactivation of the RAF gene can affect cell survival signals. Activated RAF activates the MEK/ERK signalling pathways, enabling the transmission of anti-apoptotic signals. This can lead cancer cells to resist apoptosis and continue to grow unchecked. A component of signalling pathways that regulate many biological processes, such as cell division, migration, proliferation, survival, and growth, is the ERK (extracellular signal-regulated kinase) gene. A persistent stimulation of the ERK signalling pathways, as well as an increase in cell growth and proliferation, may result from the ERK gene expansion. This could encourage the unchecked growth of cancer cells and the development of tumors. The ERK gene can alter cell survival signals if it is overactive. The transmission of anti-apoptotic signals is made possible by activated ERK, which also stimulates the cell's signalling pathways. These increases in oncogene genes showed that apoptosis was inhibited by decreasing the efficiency of caspase-3, caspase-8, and caspase-9 genes, which were increased by irisin application in melanoma cells. Inhibition of apoptosis was also confirmed by flow cytometry results. In conclusion, irisin is crucial for the identification, treatment, and prognosis of tumor malignancies. In our study, irisin hormone reduced the metastatic effects of the cells by reducing sialic acid levels in Melanoma cell line SKMEl-30 cells but had no effect on mitochondrial apoptosis.

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ODREĐIVANJE EFEKATA HORMONA IRISIN U ĆELIJAMA SKMEL-30

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Izvod

Melanociti, ćelije kože koje proizvode pigment, izvor su raka kože poznatog kao melanom. Brojne varijable, uključujući interakcije imunog sistema, mikrookruženje tumora i genetske promene, imaju uticaj na razvoj i ponašanje melanoma. Svrha ove studije je bila da se utvrdi uticaj irisina na ćelije melanoma. Za ovaj cilj su ispitivani molekularni efekti irisina SKMEL-30 na ćelije raka melanoma kod ljudi. Primenom MTT tehnike ispitani su efekti irisina na rast ćelija. RTPCR je korišćen za ispitivanje promena u nivou ekspresije gena. Koncentracije sijalične kiseline merene su spektrofotometrijom. U istraživanju je utvrđena vrednost IC50 irisina za 24-časovnu primenu na 30 nM. U poređenju sa kontrolnom grupom, nivoi sijalične kiseline u grupi SKMEL-30 ćelija tretiranoj irisinom bili su značajno niži. U qRT-PCR istraživanju, ST8SIA-2, jedan od gena glikoziltransferaze, porastao je 12,591 puta u primenjenoj grupi, dok se cas8, jedan od apoptotičkih gena, povećao 82,481 puta. U zaključku, analize protočne citometrije su pokazale da primena 30 nM irisina na ćelije SKMEL-30 utiče na proliferaciju ćelija, ali ne izaziva apoptozu. Pokazalo se da supstitucija sijalične kiseline smanjuje proliferativni i metastatski potencijal ćelija SKMEL-30.

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