



Is Irisin an Anticarcinogenic Peptide?

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Abstract

Irisin is thought an anti obesity hormone, which regulates body weight and metabolism, including insulin resistance. It is known that obesity is a risk factor in the development of cancer. This study was designed to evaluate whether there is a role of irisin on viability of human prostate cancer cells. In the present study, 0.1, 1, 10 and 100 nM concentrations of irisin were applied to human prostate cancer cells with androgen receptor positive (LNCaP) and androgen receptor negative (DU-145, PC3). Effects of irisin were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. At the end of study, all concentrations of irisin reduced viability in the three types of prostate cells, but only 10 and 100 nM concentrations of the irisin caused a significant decreases ($p<0.05$). Consequently, high concentrations of irisin in both androgen receptor positive and androgen receptor negative cell lines reduced cell viability. These results show that the cytotoxic effects of irisin on prostate cancer cells are not dependent on androgen receptor mechanism.

Key words: Irisin, LNCaP, DU-145, PC3, cell viability

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Introduction

Obesity is related with metabolic syndrome, diabetes, cardiovascular diseases and other chronic diseases [1]. Obesity also increases the risk of being susceptible to some cancer types [2]. Since a complete treatment method has not been developed so far for different cancer types, the disease increases day by day and the studies are still being conducted in this direction while this issue keeps its importance [3-6]. According to the data given in the World Cancer Report, 14.1 million new cancer cases have developed recently in the world, and 8.2 million deaths occurred because of cancer [7].

Cancer development is affected by many factors mainly by diet and hormonal factors [8-11]. The irisin, which is defined as a peptide in myokine character by Bostrom et al., is formed by the loss of 94 amino acid of the fibronectin type III domain-containing protein 5 (FNDC5) that has 206 amino acid secreted from the muscle tissue during exercise [12]. It has been reported that the gene expression of the FNDC5 is widespread in different body tissues mainly in muscles, white fat tissue, testes, epididymis, rectum, intracranial artery and pericardium [13,14]. In addition, the irisin has been shown in skeleton muscle, subcutaneous adipose tissue, heart muscle, brain-spinal cord fluid, human breast milk, saliva and purkinje cells in the cerebellum and in testicle tissue [15-17]. The most important roles of this peptide are increasing the uncoupling protein-1 (UCP1) levels, converting the white fat tissue cells into brown fat tissue cells and decreasing the obesity [12]. In our previous study, we observed that the irisin increased the body temperature and daily food consumption of the rats when it was infused intracerebroventricularly; however, despite the increasing food consumption, it was observed that the body weights of the rats did not change. These data support the assumption that the energy taken in is burnt, and the irisin significantly prevents the development of obesity [18]. The fact that the irisin encourages brown fat development, and its decreasing the body weight by increasing heat production shows that the peptide has anti obesity properties [19]. The irisin encouraging the brown fat development and decreasing the obesity, and its existence in testes and epididymis tissue and its roles in different cancer types [20] make us think that the peptide is effective in prostate cancer as well. The aim of this study was determined how the irisin would affect the cell viability in different type human prostate cancer cells (LNCaP, PC3, DU-145).

Materials and Methods

Chemicals

Penicillin-streptomycin (Biological Industries, Israel), fetal bovine serum (FBS; Sigma-Aldrich, USA), Dimetil sulfoxide (DMSO; Merck, Germany), irisin (Phoenix peptide, USA), RPMI-1640 medium and other chemicals (Sigma-Aldrich, USA) were provided. Bi-distilled water was used at all stages of the experiments. 0.1, 1, 10 and 100 nM concentrations of irisin were prepared to be used in the experiments.

Cell Culture

Androgen receptor positive (LNCaP) and androgen receptor negative (DU-145 and PC3) human prostate cancer cell lines were used in our study. All cells were fed with RPMI-1640 medium (supplemented with %10 FBS, 100U/mL penicillin and 0.1mg/mL streptomycin added) in 25 cm² culture flasks. The cells were kept in a humidified carbon dioxide incubator (%5 CO₂+%95 O₂; Panasonic, Japan) and at 37°C up to experiments.

MTT Assay

Before the irisin treatment, the viability rates of the cells were determined by using 0.4% trypan blue. If the viability rates were below 90%, we did not begin to the experiments [4,5,21]. When the cells were confluent, they were removed from the flasks by using trypsin-EDTA solution and were seeded in 96-well plates so as there were 15x10³ cells in each well. The plates were incubated for 24 h at 37°C [4,5]. The effect of the irisin on cell viability was determined by using the 3-(4,5-dimethylthiazol-2-yl)- diphenyl tetrasolium bromid (MTT) assay method [4,5,22,23]. The 0.1, 1, 10 and 100 nM concentrations of irisin to be tested were added to the wells in which the cells were placed and the cells were left in incubation for 24 h at 37°C. 0.5 mg/mL MTT working solutions were prepared from the MTT stock solution prepared in sterile PBS, and were added to the each well after than plates were incubated for 24 h with irisin. After they were kept in incubator for 3 h, the optical density of the cells were read in an ELISA reader (Synergy HT, USA) in 550 nm wavelength [4-6]. The averages of the absorbance values were taken by reading the control wells and this value was accepted as 100%. The absorbance values obtained from irisin treated wells were proportioned to the

control absorbance values, and the cell viability percentages were determined [3-5]. The assay was repeated ten times at different days [4,5,21].

Statistical Analyses

All data were presented as mean \pm SD. Normality was confirmed using Shapiro Wilk test. Homogeneity of variances was tested by Levene method. One-way analysis of variances was carried out for the comparison of the groups. For multiple comparisons, Tamhane T2 test was used owing to non-homogeneity of variances. $p < 0.05$ was considered to be statistically significant.

Results

24 h after treatment of 0.1, 1, 10 and 100 nM concentrations of irisin, the percentages of the changes (%) in viability in LNCaP cells are shown in Figure 1. Figure 2 reflects the viability percentages of DU-145 cell. In Figure 3, effects of irisin on PC3 cell viability are summarized.

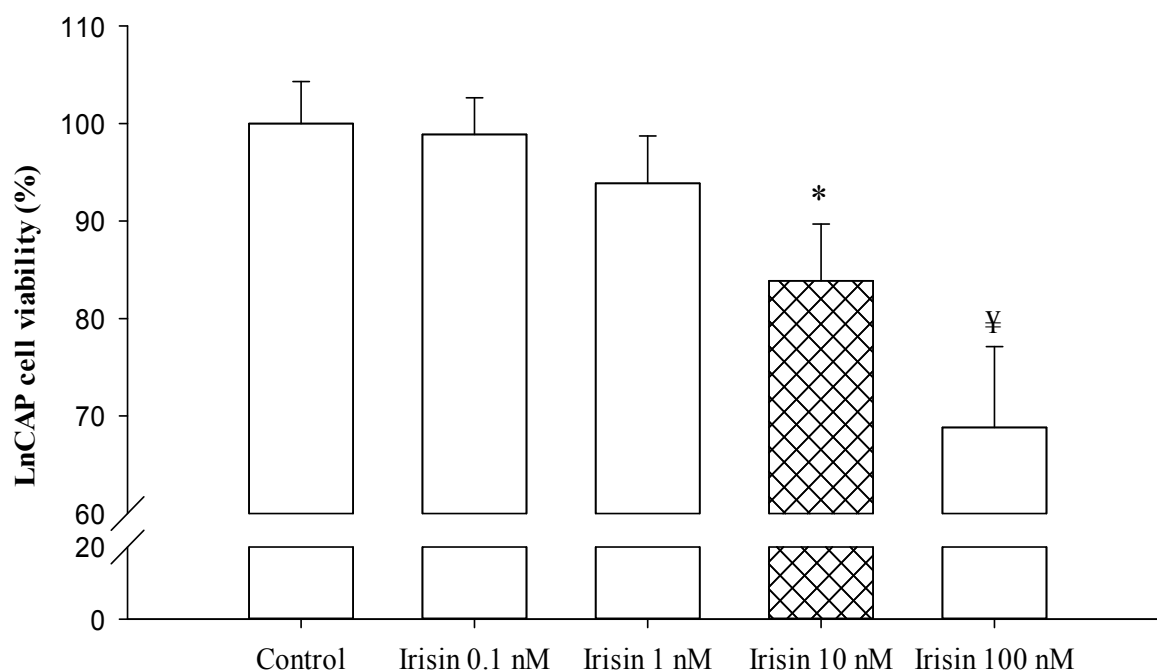


Figure 1. The viability results of LNCaP cells after treatment with irisin (Mean \pm SD; * $p < 0.05$, § $p < 0.01$; Tamhane Post Hoc, Tukey HSD).

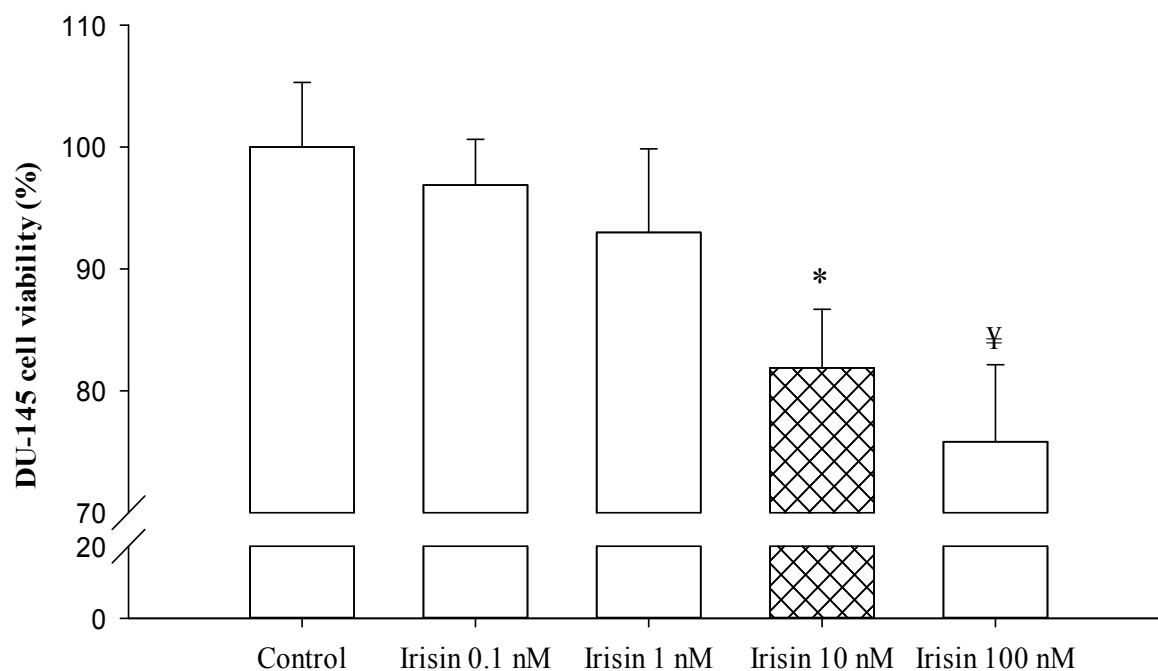


Figure 2. The viability results of DU-145 cells after treatment with irisin (Mean \pm SD; * $p < 0.05$, § $p < 0.01$; Tamhane Post Hoc, Tukey HSD).

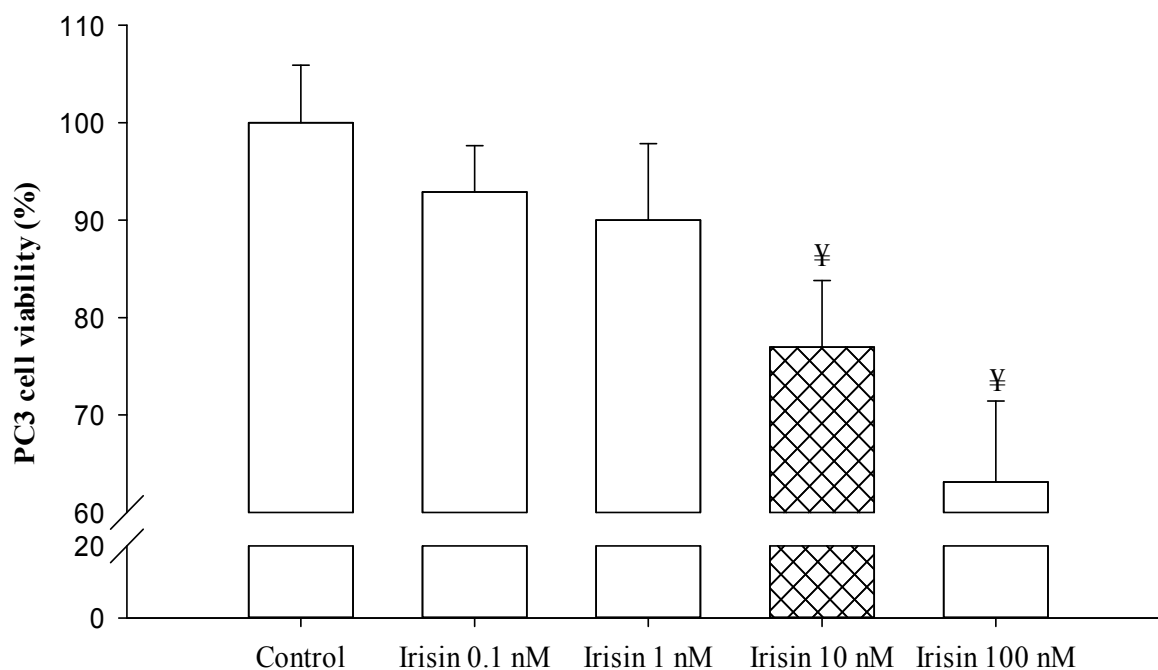


Figure 3. The viability results of PC3 cells after treatment with irisin (Mean \pm SD; § $p < 0.01$; Tamhane Post Hoc, Tukey HSD).

All treated concentrations of the irisin caused to anti-proliferative effects on LNCaP cells, but the decreases in cell viability were statistically significant only the highest doses (10 and 100 nM) of irisin ($p < 0.05$, $p < 0.01$). The application of physiological (10 nM) and pharmacologic (100 nM) concentrations of irisin caused statistically significant cell deaths both DU-145 and PC3 cells ($p < 0.05$, $p < 0.01$). In addition, the study results indicated that cytotoxic effects of irisin on different types of prostate cancer cells were dose dependent manner.

Discussion

Prostate cancer is an important health problem for men in further ages and is an important death cause depending on cancer in male individuals [25,26]. 15% of the people who were diagnosed with prostate cancer in the USA in 2010 died of this disease [27]. In Europe, it has been reported that almost half of the people diagnosed with prostate cancer die each year [28]. It is argued that testosterone and some other hormones have roles in the emergence and development of prostate cancer, and that without the effect of the hormones, the disease would not exist [29]. It is known that obesity is a risk factor in the development of cancer, and studies on the effects of some hormones that prevent obesity on cancer have become highly researched [30-33]. Irisin is secreted by muscle tissue during exercising [12] and shows wide spread in body tissues [14]. It has been shown that irisin exists not only in muscles but also in white fat tissue, liver, adult and fetal testes as well as in epididymis tissue [13,34,35]. In a study conducted by Bostrom et al., the rats were injected adenovirus particles that makes the secretion of FNDC5 possible, and it was observed that the irisin level increased 3-4 folds, and brown fat cell development accompanied with the decrease in white fat tissue [12]. Thus, irisin is defined as an anti obesity peptide. The number of studies that deal with the relation between the irisin and cancer is very few. In a study, it has been shown that the irisin application does not affect the viability of endometrial (KLE and RL95-2), colon (HT29 and MCA38), thyroid (SW579 and BHP7) and esophagus (OE13 and OE33) cancer cells [20]. In another study, it has been reported that while the treatment of irisin to human malign breast epithelial cells (MCF-10a) does not change the cell viability, it causes a decrease in the amount of viable malign aggressive breast epithelial cells [36].

In the present study, the possible cytotoxic effects of several concentrations of irisin (0.1, 1, 10 and 100 nM) on the three different types of human prostate cancer cell lines (LNCaP, DU-

145 and PC3) were determined by using MTT assay. Our study results exerted that treatment with the physiological (10nM) and pharmacological (100 nM) concentrations of the irisin significantly decreased cell viability in androgen receptor positive (LNCaP) and negative (DU-145, PC3) prostate cancer cell lines in a dose dependent manner. Additionally, the results of our study suggest that cytotoxic effects of irisin emerge via an androgen receptor independent mechanism.

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