P53 SENSITIZES HUMAN COLON CANCER CELLS TO HESPERIDIN THROUGH UPREGULATION OF BAX AND P21

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ABSTRACT

Hesperidin, a flavonoid found mainly in citrus, was reported to inhibit growth and proliferation of several cancers, including colon cancer cells. However, the question does p53 tumor suppressor protein is required for the effect of hesperidin is not yet clarified. In the present study, the effect of hesperidin on p53-expressing (HCT116 p53+/+) and p53-knockout (HCT116 p53−/−) human colon cancer cells was investigated. Hesperidin inhibits cell growth of both HCT116 p53+/+ and HCT116 p53−/− cells, however, it was more effective in p53-expressing cells. Hesperidin induced G1 cell cycle arrest in only HCT116 p53+/+ cells however induction of reactive oxygen species (ROS) and apoptosis was induced in both cells. Furthermore, hesperidin activates the proapoptotic (Bax) and cyclin dependent kinase inhibitor (p21) in only HCT116 p53+/+ cells. Interestingly, using p53 transcriptional inhibitor (pifithrin-α), hesperidin-inducing Bax and p21 upregulation in only HCT116 p53+/+ cells was reduced by cotreatment with pifithrin-α without inducing any changes in HCT116 p53−/− cells. Altogether; these results showed that hesperidin induced apoptosis and G1 cell cycle arrest in colon cancer cells in a p53/Bax - dependent and – independent , and p53/p21 - dependent

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manner; respectively.

**Keywords**: Hesperidin; Colon Cancer; HCT116; P53, Pifithrin-α, Bax, P21

**INTRODUCTION**

Hesperidin is considered as one of the most abundant flavonoids in citrus fruits such as lemons and oranges (Gil-Izquierdo et al., 2001; Aranganathan and Nalini, 2009). Hesperidin (Fig. 1) is a flavanone glycoside consisting of the flavone hesperitin bound to the disaccharide rutinose; this sugar causes hesperidin to be more soluble than hesperitin. It is also called Hesperetin 7-rhamnoglucoside, and hesperetin-7-rutinoside, and present in nature as hesperidin (glycoside form), and is deglycosylated to hesperitin (aglycone form) by intestinal bacteria prior to absorption (Ameer et al., 1996). Hesperidin has a wide range of pharmacological effects as potential anti-inflammatory, and anti-cancer drug candidate in several kinds of cells (Garg et al., 2001; Ou, 2002; Benavente-Garcia and Castillo, 2008). Also, Hesperidin has been reported to exhibit significant anti-inflammatory activity by modulating the prostaglandin synthesis and COX-2 gene expression (Hirata et al., 2005). Colon cancer is a serious health problem in most of the countries and is considered the second leading cause of cancer mortality throughout the world (Jemal et al., 2009; Jemal et al., 2010). Epidemiological studies and experimental investigations suggested factors related to socioeconomic and dietary conditions that may be important to colorectal cancer development. Significant risk factors include lower fiber intake, high fat diet and low calcium micronutrient intake may increase colon cancer incidence (Park and Conteas, 2010). Frequent consumption of fruits, vegetables and plant fiber is associated with a decrease in colorectal cancer incidence (Steinmetz and Potter, 1991; Block et al., 1992). A number of effective preventive and therapeutic natural product candidates against colon cancer have been tested to reduce both cancer incidence and mortality. The effects of hesperidin on several cancers, including colon cancer have been received a considerable research attention (Montanari et al., 1998; Park et al., 2008; Lee et al., 2010; Ghorbani et al., 2011). Hesperidin showed antioxidant effects in several cancer cells (Al-Ashaal and El-Sheltawy, 2011). On the other hand, it is stated that hesperidin induces oxidative stress and inhibits cell
proliferation of breast cancer cells (So et al., 1996; Choi, 2007; Kamaraj et al., 2010), colon cancer cells (Park et al., 2008), and prostate cancer (Lee et al., 2010). However, the precise role of p53 in the hesperidin action is not yet fully understood. In the current study, the effect of hesperidin on p53-expressing (HCT116 p53\(^{+/+}\)) and p53-knockout (HCT116 p53\(^{-/-}\)) colon cancer cells was investigated to evaluate the role of p53 in its action. To evaluate either p53 is involved or not in the action of hesperidin and does p53 is essential for induction of apoptosis and cell cycle arrest by hesperidin, we investigated the regulatory effect of hesperidin on the p53 downstream targets proapoptotic Bax and the cyclin-dependent kinase inhibitor p21 using real time PCR. Next we evaluated the role of p53 in the action of hesperidin in colon cancer cells via investigating the attenuative effect of the p53 transcriptional inhibitor pifithrin-\(\alpha\) on the cell proliferation inhibition induced by hesperidin.

**MATERIALS AND METHODS**

Chemicals and reagents: Hesperidin, Pifithrin-\(\alpha\), MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide), 2',7'-dichlorodihydrofluorescein diacetate (DCFH\(_2\)-DA), DMSO (Dimethylesulphoxide), and propidium iodide were bought from Sigma-Aldrich (St. Louis, MO, USA). RPMI cell culture media, fetal bovine serum (FBS), and penicillin/streptomycin antibiotic mixture were purchased from Gibco (Invitrogen cooperation, CA, USA). RNase-A was from USB (Cleveland, Ohio, USA). All other chemicals were obtained from standard chemical sources.

Cell lines and cell culture: Human colon cancer HCT116 p53\(^{+/+}\) (p53-expressing) and HCT116 p53\(^{-/-}\) (p53 knockout) cell lines were obtained as a gift from Mr. Kang Keusung (Department of Pathology, Kyungpook National University, Republic of Korea). Cells were originally maintained in RPMI media containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotic mixture. The cells were grown at 37\(^o\) C in a 5% CO\(_2\) incubator to be used in the following experiments. Hesperidin was also dissolved in DMSO at a final concentration of 100 mM as a stock solution.

Cell viability assay: The effect of hesperidin on the colon cancer HCT116 p53\(^{+/+}\) and HCT116 p53\(^{-/-}\) cell growth and the attenuative effect of Pifithrin-\(\alpha\) (2.5 \(\mu\)M) on the hesperidin action were
investigated using MTT cell viability assay as previously described (Park et al., 2008).

Cellular morphological changes: The morphological changes after hesperidin treatment in colon cancer HCT116 p53+/+ and HCT116 p53−/− cells were investigated as previously described (Ismail et al., 2011).

Estimation of intracellular ROS level: The effect of hesperidin on the intracellular ROS accumulation was detected using the H2O2-sensitive fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (DCFH2-DA) as previously described (Cabello et al., 2009).

Hoechst staining and nuclear fragmentation: The effect of hesperidin on the DNA damage and nuclear fragmentation of colon cancer HCT116 p53+/+ and HCT116 p53−/− cells was investigated using Hoechst staining as previously described (Ismail et al., 2011).

Trypan blue and induction of apoptosis: The effect of hesperidin on cell viability of colon cancer HCT116 p53+/+ and HCT116 p53−/− cells was investigated using trypan blue exclusion method as previously described (Shapiro et al., 1999). Trypan blue positive cells were considered as died apoptotic cell fraction.

Cell cycle analysis: The effect of hesperidin on DNA content and cell-cycle progression in colon cancer cells were estimated as previously described (Ismail et al., 2011).

Gene expression analysis: To investigate the involvement of p53 and its downstream targets in the action of hesperidin in the colon cancer HCT116 cells, real time quantitative RT-PCR analysis was performed as previously described (Ismail, 2009). The real time RT-PCR primers were designed by the Primer Express 1.5 software (Applied Biosystems) as follows: GAPDH forward, 5’-AGATCATCAGCAATGCTCCTG-3’ and GAPDH reverse, 5’-ATGGCATGGACTGTGGTCATG-3’, and Bax forward, 5’–ATGGAGCTGCAGAGGATTG-3′ and reverse, 5’–CAGTTGAAAGTTGCCATCAGCAA-3′, and p21 forward, 5’–CAAGGCCGCTCCTACATCTT-3′ and reverse, 5’–AGGAACCTCTCATTCAACCGC-3′. The expression of p21 and bax were normalized using GAPDH as a house keeping gene.

Statistical analysis: MTT, Trypan blue, and ROS data are expressed as means ± SEMs. Averages were calculated and graphed using Microsoft Excel 2003. Calculations of real time PCR data were performed by estimating the values of ∆cycle threshold (∆Ct) by
normalizing the average Ct value of each treatment compared to its opposite endogenous control (GAPDH) and then calculating $2^{-\Delta\Delta Ct}$ for each treatment and statistical analysis for data as previously described (Livak and Schmittgen, 2001). Data then were graphed and statistically analyzed by the student t-test using Sigma Plot 8.0 software.

RESULTS

Effect of hesperidin on HCT-116 cell viability

Hesperidin inhibits HCT-116 $p53^{+/-}$ and HCT116 $p53^{-}$ colon cancer cell proliferation after 24 hrs of treatment as indicated in a dose-dependent manner (Fig.1). However, there was a differential chemosensitivity between HCT-116 $p53^{+/-}$ and HCT116 $p53^{-}$ cells to hesperidin. Since these results showed that hesperidin inhibited the proliferation of $p53$-expressing (HCT-116 $p53^{+}$) cells more effectively compared to $p53$-knockout (HCT116 $p53^{-}$) cells with IC50s 55.8 and 70.8 μM, respectively (Fig.1). Interestingly, hesperidin at lower concentrations (5 μM) increased cell proliferation in only $p53$-knockout cells (Fig.1).

![Fig.1](image)

*Fig.1* The effect of hesperidin on cell proliferation of HCT116 $p53^{+/-}$ and HCT116 $p53^{-}$ colon cancer cells. Cells were treated with different concentrations of hesperidin as indicated above for 24 hrs
and then cell viability was investigated using MTT cell viability assay as shown in materials and methods. Data from three independent experiments were analyzed to determine the statistical significances using student t-test (*p < 0.01, #p < 0.05).

Effect of hesperidin on cell morphology and nuclear fragmentation

When cells were treated with hesperidin (100 µM) for 24hrs, cell shrinkage was induced, and cell number and size were reduced. Cells become rounded and lost their processes (Fig.2). In addition, hesperidin induced cell detachment and a considerable cell ratio become floating cells; showing a typical appearance of anoikis, when compared to untreated cells (Fig.2). Hesperidin was also more effective in p53 expressing cells (Fig.2).

**Fig.2** The morphological changes in human HCT116 p53+/+ and HCT116 p53−/− colon cancer cells after treatment with hesperidin. Cells were treated with hesperidin (100 µM) for 24 hrs and then the morphological changes were investigated using phase contrast microscopy.
Using nuclear Hoechst staining; we found that after treatment with hesperidin cell number decreased, nuclei showed irregular shapes, condensed with very small size, disruption of nuclear integrity and chromatin condensation was also detected with characteristic features of apoptosis (data not shown).

The effect of hesperidin on the induction of apoptosis

The apoptosis-inductive effect of hesperidin in human colon cancer HCT116 p53^{+/+} and HCT116 p53^{-/-} cell lines was investigated after treatment with different doses (0, 5, 12.5, 25, 50, and 100 µM) of hesperidin for 24 hrs using trypan blue exclusion method and examined using phase contrast microscopy. Total number of died cells were counted as those took up trypan blue dye and appeared blue color while viable cells are those excluded the trypan blue dye and appeared with clear cytoplasm. Hesperidin induced cell death and reduced cell viability in both HCT116 p53^{+/+} and HCT116 p53^{-/-} cells (Fig.3). Hesperidin showed no effect at low doses (5 µM) in both cells and then showed increased effect at higher doses (12.5, 25, 50, and 100 µM) in a dose-dependent manner (Fig.3). Also, hesperidin was more effective in p53-expressing cells compared to p53-knockout cells (Fig.3).

![Fig.3](image_url)  

**Fig.3** The effect of Hesperidin on cell death and induction of apoptosis in HCT116 p53^{+/+} and HCT116 p53^{-/-} colon cancer cells was determined using trypan blue exclusion method. Cells were treated with hesperidin as indicated for 24 hrs and then harvested and stained
with trypan blue and examined using phase contrast microscope. Total number of died cells were counted by trypan blue exclusion method and these results are averages of three independent experiments. Statistical differences were investigated using student t-test (*p < 0.01).

The effect of hesperidin on the intracellular reactive oxygen species (ROS)

The effect of hesperidin on the cellular release of ROS was investigated by measuring the changes in the fluorescence using DCFH-DA. Hesperidin treatment markedly increased the DCFH-DA derived fluorescence at high concentrations (≥ 25 μM) in both cells, and reduced at low concentrations (≤ 5 μM) in only p53-knockout cells (Fig.4).

This hesperidin-mediated increase in ROS release was preferentially higher in p53-knockout cells (HCT116 p53−/−) compared to the p53-expressing cells (HCT116 p53+/+) (Fig.4). This high increase in p53-knockout cells may due to the finding that the basal ROS level is higher in p53-knockout cells compared to p53-expressing cells (Ismail et al., 2011).

Fig.4 The effect of hesperidin on the intracellular reactive oxygen species (ROS) in HCT116 p53+/+ and HCT116 p53−/− colon cancer cells. Cells were treated with hesperidin as the indicated concentrations for 24 hrs and then incubated with 5 μg/ml of DCFH-DA for 30 minutes and then processed for measuring ROS level as
indicated in materials and methods. Data from three independent experiments were analyzed to determine the statistical differences using student t-test (*p < 0.01, #p < 0.05).

The effect of hesperidin on cell cycle progression

Cells were treated with different concentrations of hesperidin (25, 50, 100 µM), and 0.1%-treated DMSO as a control for 24 hrs as indicated in materials and methods. In p53-expressing (HCT116p53\textsuperscript{+\textordmasculine}) colon cancer cells, hesperidin reduced cells at S and G2\textasciitilde M phase and accumulated cells at G1 phase (Table 1), and the accumulation of cells as G1 phase increased after hesperidin treatment in a concentration-dependent manner (Table 1). Also, hesperidin induced apoptosis in HCT116p53\textsuperscript{+\textordmasculine} cells in a concentration dependent manner (Table 1).

However under the same experimental conditions, in p53-knockout (HCT116 p53\textsuperscript{-\textordmasculine}) cells, hesperidin showed no effect on cell cycle progression at all concentration used in this experiment (Table 1). Also, hesperidin induced apoptosis in HCT116 p53\textsuperscript{-\textordmasculine} cells without affecting cell progression distribution.

The effect of hesperidin on Bax and p21 expression

To test the role of p53 in the action of hesperidin-inducing cell proliferation inhibition, we investigated the effect of hesperidin on the p53 activation through investigation of its downstream targets involved in apoptosis (Bax) and cell cycle arrest (p21). Interestingly, hesperidin induced bax and p21 mRNA expression levels in only p53-expressing HCT116 p53\textsuperscript{+\textordmasculine} cells without showing any effect in p53-knockout HCT116 p53\textsuperscript{-\textordmasculine} cells (Fig. 5A & 5B). These data suggest that hesperidin-inducing Bax and p21 takes place via activation of p53. To confirm these data we blocked p53 transcriptional activity in both cells via using p53transcriptional inhibitor pifithrin-α, and then investigate the effect of hesperidin on bax and p21 transcriptional activation. Interestingly, pifithrin- α was able to repress only p21 transcription without affecting Bax expression in only HCT116 p53\textsuperscript{+\textordmasculine} cells. Consistently, we found that, pifithrin-α was able to significantly inhibit the Bax and p21 transcription induced by hesperidin in only HCT116 p53\textsuperscript{+\textordmasculine} cells without any significant changes in HCT116 p53\textsuperscript{-\textordmasculine} cells (Fig. 5A & 5B).
**Fig. 5.** The effect of hesperidin on the proapoptotic Bax (A) and cyclin dependent kinase inhibitor p21 (B) transcriptional levels were investigated using real time PCR. Cells were treated with either 100 µM of hesperidin (H-100), pifithrin-α (2.5 µM), or cotreatment of hesperidin (100 µM) and pifithrin-α (H-100-α) in HCT116 p53\(^{+/+}\) and HCT116 p53\(^{-/-}\) colon cancer cells for 24 hrs. For cotreatment, cells were first pretreated with pifithrin-α for 30 minutes followed by treatment with pifithrin-α. Bax and p21 mRNA expressions were then evaluated using real time PCR. Data from three independent experiments were analyzed and statistical significances were determined using student t-test (*p < 0.01, #p < 0.05).

The effect of pifithrin-α on the cell viability inhibitory action of hesperidin

Under the same experimental conditions as in Fig.5, the attenuative effect of pifithrin-α on the antiproliferative activity of hesperidin was investigated via cotreatment of pifithrin-α and hesperidin for 24 hrs as shown in materials and methods. pifithrin-α (2.5 µM) was pretreated for 30 minutes followed by cotreatment with hesperidin- pifithrin-α for 24 hrs, and then cell viability assay was investigated using MTT assay. pifithrin-α significantly reduced the cell proliferation inhibitory effect of hesperidin in only HCT116 p53\(^{+/+}\) cells without any significant additive or recovery role in HCT116 p53\(^{-/-}\) cells (Fig.6).
**Fig. 6.** The attenuative effect of pifithrin-α on cell proliferation inhibition induced by hesperidin in HCT116 p53<sup>+/+</sup> and HCT116 p53<sup>-/-</sup> human colon cancer cells was assessed using MTT cell viability assay. Cells were treated with 100 µM of hesperidin (H-100), or pretreated for 30 minutes with pifithrin-α and/or followed by 24 hrs cotreatment with hesperidin- pifithrin-α (H-100-α) as indicated and then cell viability was measured. Data from three independent experiments were analyzed and statistical differences were investigated using student t-test (*p < 0.01).

**Table 1.** The effect of Hesperidin (hesp) on cell cycle progression in HCT116 p53<sup>+/+</sup> (Fig. 6.A) and HCT116 p53<sup>-/-</sup> (Fig. 6.B) colon cancer cells using flow cytometric analysis. In p53 expressing (HCT116 p53<sup>+/+</sup>) cells hesperidin reduced cells at S and G2/M phase and accumulated cells at G1 phase; however, in p53 knockout (HCT116 p53<sup>−/−</sup>) cells; hesperidin showed no effect on cell cycle progression at all concentrations used in this experiment.

<table>
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<th>Hesp 50 µM</th>
<th>Hesp 100 µM</th>
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<td>21</td>
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<td>G2M</td>
<td>25.3</td>
<td>22.3</td>
<td>17.8</td>
<td>14.7</td>
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<tr>
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<td>51.8</td>
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DISCUSSION

According to the World Health Organization (WHO), about 1.1 million new cases diagnosed with colorectal cancer in 2010. This estimation is consistent with the study that over one million new cases of colorectal cancer estimated to be added in 2010 (Jemal et al., 2009). Recent studies suggest that there is a close relationship between cancer incidence and our nutritional behavior and nutrient components (Ames et al., 1995). Also, several epidemiological studies of colorectal cancer suggested that dietary agents play an important role in the development of intestinal neoplasia (Ames et al., 1995). Here we investigated the effect of hesperidin on human colon cancer p53-expressing (HCT116 p53^{+/+}) and p53-knockout (HCT116 p53^{-/-}) cells. Hesperidin has many biological activities; including anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant effects and decreasing capillary fragility (Garg et al., 2001). Our data showed that, hesperidin inhibits cell proliferation differentially in p53-expressing and p53-knockout HCT116 cells. Proliferation inhibitory action induced by hesperidin was more effective in p53-expressing (HCT116 p53^{+/+}) cells despite the release of reactive oxygen species (ROS) in both cells after hesperidin treatment, particularly at higher concentrations. This ROS release is followed by DNA damage, chromosomal condensation, and nuclear fragmentation. Also trypan blue exclusion method showed that hesperidin induced cell death and apoptosis in both cells. Furthermore, consistently to our results, it is reported that hesperidin inhibits cell proliferation and induced apoptosis via bax and caspase 3 activation in p53-expressing human colon cancer SNU-C4 cells (Park et al., 2008). On the other hand, hesperidin showed strong antioxidant properties and less cytotoxicity to the B16 mouse melanoma cells (Zhang et al., 2007). It is reported also that, hesperidin has powerful protection effects on DNA damage, reducing the frequency of micronuclei induced by γ-irradiation in mouse bone marrow cells (Hosseinimehr and Nemati, 2006).

The tumor suppressor p53 protein plays important roles in a diversity of physiologic functions. Cellular p53 functions as a tumor suppressor by increasing genomic stability and inhibiting cell transformation. In accordance with our results of the regulatory effect of hesperidin on
p53, it is stated that hesperidin induced p53 and inhibits NF-kB activation to induce apoptosis in NALM-6 cells via Bax activation and caspase 3 cleavage (Ghorbani et al., 2011). After DNA damage active p53 works with p21 to induce cell cycle arrest (Bunz et al., 1998; Fuster et al., 2007). This is consistent with our data that, in only p53-expressing (HCT116 p53<sup>+</sup>) cells, hesperidin induced G1 cell cycle arrest. Under the same experimental conditions, hesperidin induced p21 expression in only HCT116 p53<sup>+</sup> cells, suggesting that G1 cell cycle arrest-induced by hesperidin takes place in a p53/p21-dependent manner. The p53-regulated pro-apoptotic function is believed to contribute to the efficacy of the anti-cancer therapy to enhance cellular chemosensitivity via inducing p53-dependent growth inhibition (Fuster et al., 2007). Currently, we found that hesperidin induced apoptosis in both cells while activated Bax expression in only HCT116 p53<sup>+</sup> cells, suggesting that bax activation is not required for hesperidin-inducing apoptosis in HCT116 colon cancer cells.

Pifithrin-α, a potent and specific inhibitor of p53, the protective effects of pifithrin-α involved both down-regulation of the transcriptional activation of Bax and a direct inhibition of p53 translocation to mitochondria (Dagher, 2004). The effects of pifithrin-α on p53 includes the inhibition of the transcriptional activation of the p53 downstream targets such as p21 and Bax and inhibition of p53 translocation to the mitochondria. These data were also supported by knowing that pifithrin-α inhibits p53 function in vivo by blockade its translocation to the nucleus and mitochondria (Leker et al., 2004). Also, pifithrin-α exerts an anti-apoptotic effect by inhibiting not only Bax but also by inhibiting the p53-regulated pro-apoptotic gene PUMA (Luo et al., 2009). In the current data, pifithrin-α treatment recovered cell growth inhibition induced by hesperidin in only HCT116 p53<sup>+</sup> cells. Therefore, in the present study we found that pifithrin-α inhibits hesperidin action via inhibiting p53-inducing Bax and p21 upregulation mediated via hesperidin treatment in HCT116 p53<sup>+</sup> cells.

Altogether, it could be concluded that, p53 is not essential for hesperidin-inducing apoptosis but essential for G1 cell cycle arrest in HCT116 colon cancer cells. Also hesperidin induced Bax-independent apoptosis in the absence of p53. These data suggest that, p53 has the ability to sensitize and increase HCT116 cellular chemo-sensitivity to hesperidin via inducing G1 cell cycle arrest and Bax-dependent...
apoptosis. Also hesperin failed to activate bax and p21 in the absence of p53. Finally, these data suggest that hesperin inhibits colon cancer cell growth via several pathways including; p53/p21-dependent G1 cell cycle arrest, and p53/Bax-dependent apoptosis as well as p53/bax-independent apoptosis (Fig.7).

**Fig.7.** Schematic diagram shows the action and pathways involved in the action of hesperidin in colon cancer HCT116 cells. Hesperidin inhibits HCT116 cell proliferation in a p53-dependent and – independent manners with differential chemosensitivity.

**REFERENCES**


الملخص العربي

يرى البروتينات P53 و P21 تأثيرها في استجابة خلايا سرطان القولون للهسيدين عن طريق زيادة الـ BAX

- باسمعيل أحمد اسماعيل، محمد سيد جبرى، شيماء قطب، منى عبد الرحمن إبراهيم.: معالج بيولوجيا الخليج البطنية - قسم علم الحيوان - كلية العلوم - جامعة أسوان. 

- قسم علم الحيوان - كلية العلوم - جامعة أسوان.


P53

وقد أوضحت الدراسة أن الهسيدين يعزز زيادة شقائق الأكسجين الحرة (ROS) في الخلايا وتقليل من الخلايا مما يبين أن الهسيدين له P53 P53 P53 (ROS (apoptosis (apoptosis)

القدرة على تخفيض الموت المبرمج لخلايا سرطان القولون دون الحاجة لبروتينات الخلايا المنقولة.)

P53

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P53

P53/Bax-independent pathway P53

P53/Bax dependent pathway P53

p53/Bax independent pathway

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p53/Bax dependent pathway

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