

**GENISTEIN DIMINISHED CIZ1 EXPRESSION IN DAUDI LYMPHOID CELLS.**

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**ABSTRACT :** Cip-interacting zinc finger protein 1 (Ciz1), stimulates DNA replication and has been implicated in tumorigenesis of breast cancer cells. In order to investigate the possibility of using medicinal isoflavone against breast cancer, we studied whether some isoflavones could affect the expression of the Ciz1. The *in vitro* effect of isoflavone treatment on the reduction of Ciz1 expression was detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Western blotting also confirmed the down-regulation of the protein at dose dependent manner of the genistein treatment in Daudi lymphoid cell line cells.

**Keywords:** isoflavone, genistein, gene expression, Ciz1, breast cancer

**INTRODUCTION**

Breast cancer is the most commonly diagnosed cancer in women and is a major cause of cancer deaths (Jemal A, et. al., 2008). A large number of previous works have provided insight about the roles of cell cycle components in transcriptional stimulation by estrogen in the breast cancer cells. The transcriptional activity of the estrogen receptor (ER) is affected by regulatory cofactors including coactivators and corepressors. Among them, Ciz1 stimulates DNA replication depending on ER and participates in the regulation of cell cycle by increasing cdk2 kinase activity and inducing the G1-Transition (den Hollander P, et. al., 2006). Ciz1 protein coregulates ER by enhancing its transactivation activity and recruitment to target gene chromatin. Hypersensitivity to estrogen has been observed in patients with breast cancer (Santen RJ, et. al., 2005). Ciz1 induces such the hypersensitivity of breast cancer cells to estrogen and induces the expression of ER target gene cyclin D1. Then, overexpression of Ciz1 promoted the growth-rate, anchorage independence and tumorigenesis of breast cancer cells (den Hollander P, et. al., 2006). Accordingly, repression of Ciz1 expression may be expected to have interference effects on breast cancer progression.

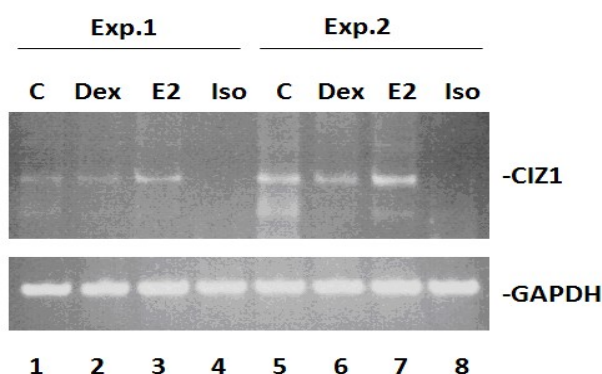
Bio-properties of dietary soy have been suggested in numerous studies. Isoflavones present in soy have been shown to be bioactive compounds. When significant quantities of isoflavones are consumed, their bioactivity has been proposed to favorably affect blood lipid levels, symptoms of menopause, and osteoporosis (Demonty I, et. al., 2003, Zhan S, et. al., 2005). Isoflavones may exert an effect at least in part by modifying the activity of certain factors. For example, isoflavones have been shown to affect metabolisms via the activation of PPAR (Dang ZC, et. al., 2003, Kim S, et. al., 2004,) and affect the expression of HMG-CoA reductase through sterol regulatory element binding protein regulation in HepG2 cells (Mullen E, et. al., 2004). The protective effects of soy observed with respect to cardiovascular disease and bone and menopausal health may in part be due to the regulation of transcriptional expression of certain enzymes (Setchell KD, et. al., 2002).

Investigation of variations in gene expression as a result of isoflavone treatment might help define the underlying mechanisms of soy actions. We then hypothesized that some isoflavones could affect the expression of the Ciz1.

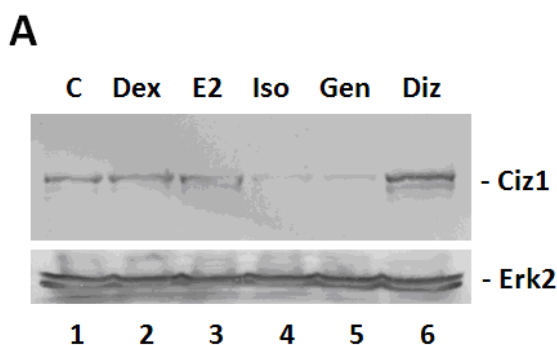
## RESULTS AND DISCUSSION

In order to investigate the possibility of using medicinal phytochemicals, isoflavone, estradiol, as well as dexamethasone were added into cell culture medium of K562, Jurkat or Daudi cells and the levels of Ciz1 expression were examined. Aglycone form of isoflavone was used for the experiment. We first employed RT-PCR analysis to quantify the expression level of Ciz1 gene. Total RNA was isolated 24 hr after treatment for detection of Ciz1, and the levels of mRNA were determined by the conventional RT-PCR. As shown in Figure 1, the Ciz1 gene expression level greatly decreased in the treatment of isoflavone at the final concentration  $10^{-5}$  M, compared with the untreated ethanol vehicle, estradiol and dexamethasone, in the Daudi cells. On the contrary, the expression of the housekeeping gene GAPDH was unaltered. There was almost no difference on the results of gene expressional profile between Daudi and Jurkat cells, and no reduction of Ciz1 expression in K562 cells. To exclude the possibility of carry-over contamination, reactions containing all RT-PCR reagents including primers without sample RNA were preformed as negative controls. No such RNA contamination was detected (data not shown).

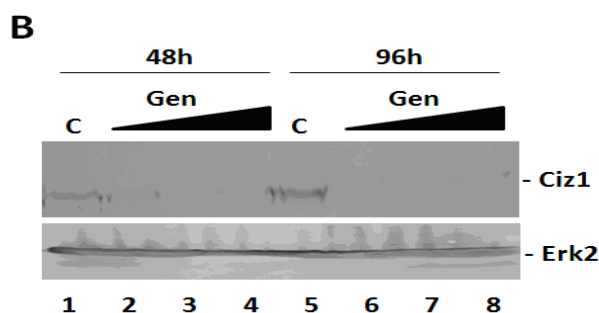
To further confirm the expression status of Ciz1 reduced by the isoflavone, western method was also performed to analyze the level of Ciz1 protein in the Daudi cells. As shown in Figure 2A, the isoflavone, genistein, but not daidzein treatment also diminished the protein expression of Ciz1. This protein expression profile approximately agreed with the result of RT-PCR shown in Figure 1. We then addressed a question whether the genistein can reduce Ciz1 expression at dose dependent manner. After pre-treating the cells with a set of different dose of concentrations of the genistein, we found that Ciz1 protein expression was decreased with the increasing concentrations of the genistein. As shown in Figure 2B, final concentration  $10^{-6}$  M l of the genistein diminished the Ciz1 expression by more than 90% in the Daudi cells after 96 hr genistein stimulation.



**Figure 1.** Ciz1 mRNA was analyzed by semi-quantitative RT-PCR. The semiquantitative RT-PCR was performed using primers specific to Ciz1 (Fw : 5'-ACATATCCACAGGTCCACACAC-3', Rv : 5'-CTGCTCATGGGTCTGCTCTG-3') or GAPDH control (Fw: 5'-TCCCATCACCATCTTCCA-3', Rv : 5'-CATCACGCCACAGTTTCC-3') on 100 ng total RNA prepared from Daudi cells treated without (lane 1, lane 5) or with dexamethasone (SIGMA) (lane 2, lane 6), estradiol (SIGMA) (lane 3, lane 7), isoflavone (INDOFINE chemical) (lane 4, lane 8) at the final concentration  $10^{-5}$  M for 24 hr. The Daudi cells were maintained in RPMI1642 supplemented with 10 % fetal bovine serum, penicillin and streptomycin at 37°C in a humidified atmosphere containing 5 % CO<sub>2</sub>. Aglycone form of isoflavone was used for the experiment. Specific expression was determined in relation to the expression of the housekeeping gene GAPDH used as an internal loading control. At least four independent experiments were done, and typical two paired results were documented.



**Figure 2A.** Genistein diminished the expression of Ciz1 protein. Daudi cells were treated without (lane 1) or with dexamethasone (lane 2), estradiol (lane 3), isoflavone (lane 4), genistein (Cayman Chemical) (lane 5), daidzein (Cayman Chemical) (lane 6) at the final concentration  $10^{-5}$  M for 48 hr. After treatment, cell lysates were isolated, the levels of Ciz1 protein was detected by western blot analysis using anti-Ciz1 antibody (COSMO BIO). Western blot with anti-Erk2 antibody (BD Bioscience) was also shown as equal levels of protein loading.



**Figure 2B.** Dose dependent repression of Ciz1 protein expression. Daudi cells were treated without (lane 1, 5) or with genistein at the final concentration  $10^{-6}$  M (lane 2, 6),  $10^{-5}$  M (lane 3, 7),  $10^{-4}$  M (lane 4, 8) for 48 hr (lane 1, 2, 3, 4) or 96 hr (lane 5, 6, 7, 8). The levels of Ciz1 protein were detected by western blot analysis using anti-Ciz1 antibody as figure 2A. Western blot with anti-Erk2 antibody was also shown as equal levels of protein loading.

Many of phytochemicals present in a wide variety of plants might preferentially interact with ER among the hormone receptors belonging to the nuclear receptor superfamily (Takeuchi S, et. al., 2009). There are a number of *in vitro* studies of the estrogenic properties specially genistein and daidzein. (Mueller S. O., et. al., 2004, Harris D. M., et. al., 2005) These are structurally similar to estrogen and genistein and daidzein, but not glycitein, can bind to and transactivate ER (Chrzan BG, et. al., 2007, Sakamoto T, et. al., 2010). Soy isoflavones are present in soy foods as aglycones where genistein, daidzein, and glycitein make up 50%, 40%, and 10%, respectively, of the total soybean isoflavones (Murphy PA, et. al., 1999). Recent data indicate that genistein inhibits invasion, metastasis, and angiogenesis *in vitro* and *in vivo* in a number of cancers including breast cancer (Singh AV, et. al., 2005, Vantuyghem SA, et. al., 2005). However, other studies have shown that daidzein increased while genistein decreased mammary tumor growth compared to vehicle, respectively (Martinez-Montemayor MM, et. al., 2010). Moreover, the contradictory result that genistein increased the growth of MCF-7 human breast cancer cells and tumors in ovariectomized nude mice and metastatic progression of prostate cancer has also been reported (El Touny LH, et. al., 2009, Jeng YJ, et. al., 2009).

Genistein affects cellular function via inhibition of tyrosine protein kinases, cyclooxygenase 2 and cytochrome p450 enzymes (Rice S, et. al., 2006, Shon YH, et. al., 2006). Genistein also modulates ER levels, the activity of topoisomerase II, enzymes involved in phospho-inositide turnover, mitogen activated protein kinases and NF-kappaB signaling pathways (Singh AV, et. al., 2006, Rabiau N, et. al., 2010). Here, we show that genistein is the potent transcriptional repressor for Ciz1 gene. The precise mechanism of transcriptional regulation of Ciz1 by genistein remains unclear. The Ciz1 gene might be complicatedly regulated by various transcription factors. More studies including *in vivo* experiments need to be undertaken to elucidate the molecular mechanisms of the isoflavone.

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**Competing interests statement:** The authors declared that no conflict of interest exists.

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