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Estrogenic Activity of some Phytoestrogens on Bovine Oxytocin and Thymidine Kinase-ERE Promoter through Estrogen Receptor-α in MDA-MB 231 Cells

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Abstract

Background: Phytoestrogens, a group of plant-derived polyphenolic compounds have recently come into considerable attention due to the increasing information on their potential adverse effects in human health**.** Some of phytoestrogens show estrogenic activity that may be carcinogenic for human. In the present study here, we investigated the transcriptional effects of variety of phytoestrogens on the bovine oxytocin and the thymidine kinase-ERE promoter by estrogen receptor α in MDA-MB 231 breast cancer cell line.

Materials and Methods: Cells were seeded for transfections into 12- well plates at a density of 100000 cells per well and were transfected with a total of 3 μg of plasmid DNA using calcium phosphate coprecipitation. Estrogen and some phytoestrogens (naringenin, 8-prenyl-naringenin and 6-(1, 1-dimethylallyl) naringenin were used for stimulation of transfected cells.

Results: Findings of our study clearly demonstrated the subtype-selective activation of estrogen receptor (ER)α and (ER)β by the phytoestrogen naringenin (activating estrogen receptor β) and its substituted forms 8-prenyl-naringenin and 6-(1, 1-dimethylallyl) naringenin (activating estrogen receptor α), on the ERE-controlled promoter as well as on the oxytocin gene promoter.

Conclusion: The study revealed that some phytoestrogens show estrogenic activity by classical or non-classical mechanisms as well as exhibit estrogenic activity by undetermined mechanisms in transfected MDA-MB 231 cell line.

Keywords: Phytoestrogens; Naringenin; Oxytocin; Transfection

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Introduction

Regulation of gene transcription is involved in the control of cell growth, proliferation, and differentiation (1). As result of activation of estrogen receptor, some natural compounds produced by plants are referred to as phytoestrogens and have the potential to interrupt or modulate the endogenous estrogenic signalling (1). Phytoestrogens are compounds that are naturally presented in edible plant material. They are constituents of many human food stuffs such as beans, peas, sprouts cabbage, spinach, soybean, and hops. They contain classes of compounds such as the isoflavones, lignanes, and comestans (2, 3). They are present to varying extents in the diet. More recently, multiple epidemiological investigations have demonstrated a relationship between high dietary intake of isoflavones and lignans and lower rates of certain cancers, cardiovascular problems, and menopausal symptoms (4). It was also known that phytoestrogens could compete with estradiol for binding to intracellular estrogen receptors (5). Phytoestrogens reveal pleiotropic effects on cellular signalling and show some favourable effects on estrogen-dependent disorders (1). The phytoestrogens contained in the diet and in food supplements have the potential for both risks and benefits with respect to human health. Current research suggests that phytoestrogens may be natural "Selective Estrogen Receptor Modulators" (SERMs) (3), which means that they can bind to

certain estrogen receptors in some tissues, either activating or down-regulating cellular responses. There has been great interest in the possibility that dietary phytoestrogens may be an alternative to postmenopausal hormone therapy, due to concerns about side effects and long-term health consequences that prevent many women from using hormone herapy for improvement of the disease associated with the menopausal transition (6). Estrogen-like molecules act predominantly through estrogen mediated activation of transcription via estrogen responsive elements (ERE). Both estrogen receptor α and estrogen receptor β can interact with various cell cycle transcriptional factors (7), but the molecular mechanisms of phytoestrogens effects are not well understood. Satih *et al* studied the estrogen-like effects of phytoestrogens on the ER that employed two types of cell lines: ER+ cell lines (MCF-7) and ER- cell lines (MCF-10a and MDA-MB-231). They identified 278 and 334 differentially expressed genes after genistein or daidzein treatment, respectively, in estrogen-positive (MCF-7) and estrogen-negative (MDA-MB-231, MCF-10a) cells (8). Hirvonen *et al* investigated whether phytoestrogens modulated the transcriptional activity of ERRγ. They demonstrated that the phytoestrogen equol acts as an ERRγ agonist (9). The study presented here now aims at the elucidation of classical (bind to its nuclear receptor) and non-classical mechanisms (act via other mechanism than attachment to its nuclear receptor) by a detailed analysis of the components of the regulatory system controlling the oxytocin promoter. Transient transfection experiments using the breast cancer cell line MDA-MB 231 were performed and are presented here.

Materials and Methods

Cell Culture

MDA-MB 231 breast cancer cell line was used because it has shown to be devoid of functional estrogen receptor α and estrogen receptor β. MDA-MB 231 cells were plated out in small 25 cm^2 culture flasks (Nunc, Wiesbaden, Germany) in phenol red free Dulbecco's modified Eagle's medium (DMEM) (Gibco, Karlsruhe, Germany) supplemented with 10% of steroid-free (charcoal-stripped) fetal calf serum (CCS) plus 1% of L-glutamine (Sigma, Taufkirchen, Germany) and 0.5% of penicillin/ streptomycin (Sigma) and incubated at 37 °C / 5% CO2.

Before reaching confluence, cells were detached from the bottom of the flask by the addition of 1.5ml 1x Viralex TM-EDTA solution (0.05% trypsin, 0.5 mM EDTA) followed by incubation at 37 °C for 2 min. Cells were harvested by centrifugation for 2 minutes at 6500 g and resuspended in culture medium. Resuspended cells were re-plated either into another flask (75cm2) at one-third of the density or into 12-well plates (Nunc) at a density of 100000 cells per well for transfection. In addition, MCF-7 cells were applied as control.

Estrogen and phytoestrogens

17ß-estradiol (Sigma-Aldrich), Naringenin (Sigma-Aldrich), 6-(1, 1-dimethylallyl)-naringenin (6DMA-naringenin) (Sigma-Aldrich) and 8-prenylnaringenin (8p-naringenin) (Sigma-Aldrich) were used for stimulation of transfected cells.

Transfection Assays

In each well of 12-well plates (Nunc), 10^5 MDA-MB 231 were seeded and transfected on the next day with 3 µg of plasmid DNA using calcium phosphate coprecipitation (Profection Mammalian Transfection System, Promega, Mannheim, Germany). The transfected plasmid DNA included 1µg of promoter-luciferase plasmid, 1.5µg of the relevant ER expression vector and 0.5µg of a LacZ control vector driven from the CMV early promoter. Following transfection, cells were incubated for 16 h, then medium was changed, and cells were stimulated with estrogen and phytoestrogens in 2.5 ml fresh culture medium per well. In the control group estrogens and phytoestrogens did not add. Twenty-four hours after stimulation, cells were washed two times with phosphate-buffered saline and then 100 µl of Lysis Buffer (Promega) were added and the plates incubated at room temperature for 5 min with slow shaking.

The cell lysates then were scraped off the plates with cell scrapers and transferred to Eppendorf tubes on ice. After centrifugation for 1 min at maximum speed in an Eppendorf microfuge, the supernatants were transferred to new tubes and used immediately for determination of luciferase and β-galactosidase activity. For measuring luciferase activity, the Firefly Luciferase Reporter Gene Assay System (Promega) was used according to the manufacturer's instructions. Twenty microliters of cell lysate were added to 100 µl of Luciferase Assay Reagent and the luminescence measured for 5 s with 2 s delay time in a Berthold Sirius luminometer. β-galactosidase activity was measured using the Galacto-Light assay system (Tropix, Bedford, MA, USA) following the protocol supplied with the kit. Five microliters of cell lysate were incubated with 200 µl of diluted substrate for 1 h at room temperature.

Then 300 µl of accelerator solution were added and the luminescence measured as above. After determination of luciferase and β-galactosidase activities of each single cell lysate, the transcriptional activities of the promoters under investigation were normalized by dividing the luciferase values by the β-

galactosidase values to control for transfection efficiency, resulting in arbitrary so-called relative light units (10).

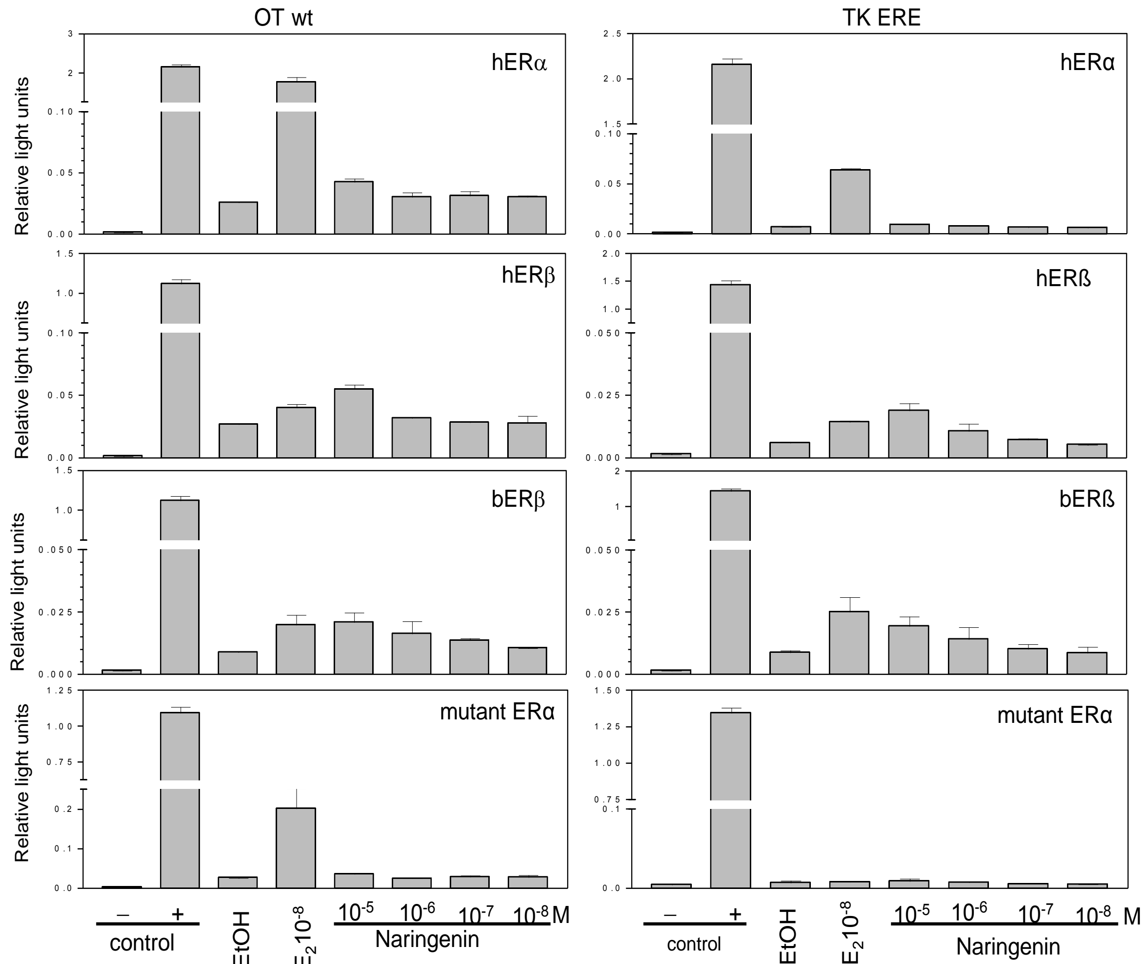

Figure 1. Transcriptional activity of naringenin on the oxytocin promoter and the thymidine kinase-ERE promoter by different estrogen receptors in the MDA-MB 231 cells. MDA-MB 231cells were transfected with the oxytocin promoter construct (OTwt) or the thymidine kinase-ERE promoter construct (TK ERE) and human estrogen receptor α (hERα) human estrogen receptor ß (hERß), bovine estrogen receptor ß (bERß) or estrogen receptor α mutant unable to bind DNA (mutant ERα) and treated with different concentrations of naringenin.

DNA constructs

All promoter-reporter constructs were as previously described (10). Either the bovine oxytocin promoter (-183 to +17) was used (OXT), inserted into the pGL3-Basic vector (Promega), or the thymidine kinase promoter controlled by a single vitellogenin ERE (TKERE) was used to drive a similar luciferase reporter construct (11) As negative and positive controls, respectively, we used the pGL3-Basic plasmid, containing neither promoter nor transcriptional enhancer sequences, and the pGL3-Control vector, expressing luciferase under the control of the SV40 promoter and enhancer (both from Promega). Estrogen receptor (ERα) was generated from an expression construct comprising the human ERα cDNA controlled by a CMV viral promoter (10). A version of estrogen receptor (ERα) where in the DNA-binding domain (DBD) was mutated so that the receptor was no longer able to interact directly with an ERE as in Koohi *et al* (10). A human ERα (hERα) expression vector was obtained as a generous gift from Dr. Katrin Stedronsky (Institute for Hormone and Fertility Research, Hamburg, Germany) and the bovine ERα (bERα) expression construct was prepared as in Walther *et al* (10) and driven from a CMV promoter.

Cell Toxicity Assay

All compounds used were additionally tested for any cytotoxic effects at the concentrations used, which could influence the results obtained. Firstly, cells were checked for integrity following 24 h incubation with the test substance by staining washed cells for 3 h with 2% neutral red, and after further washing in

PBS, photometrically measuring the absorbed dye at 540 nm. Secondly, following exposure to the test in the flasks were stained with 10% Giemsa (Merck) and cells counted.

Statistical analysis
All experiments were performed in duplicate or triplicate dishes and each experiment was repeated at least three times. The mean ± SD was calculated for all stimulation experiments. Following correction for substances, cells were replated into T75 flasks, and subjected to 7 days of culture, after which all colonies transfection efficiency, results were expressed as relative light units (means + S.D. for triplicate wells). Statistical significance for the differences in promoter activities was assessed by one way ANOVA followed by the Newman-Keuls test, or unpaired t-tests with Welch's correction using the GraphPad Prism 3.0 software package (GraphPad Software Inc., San Diego, CA).

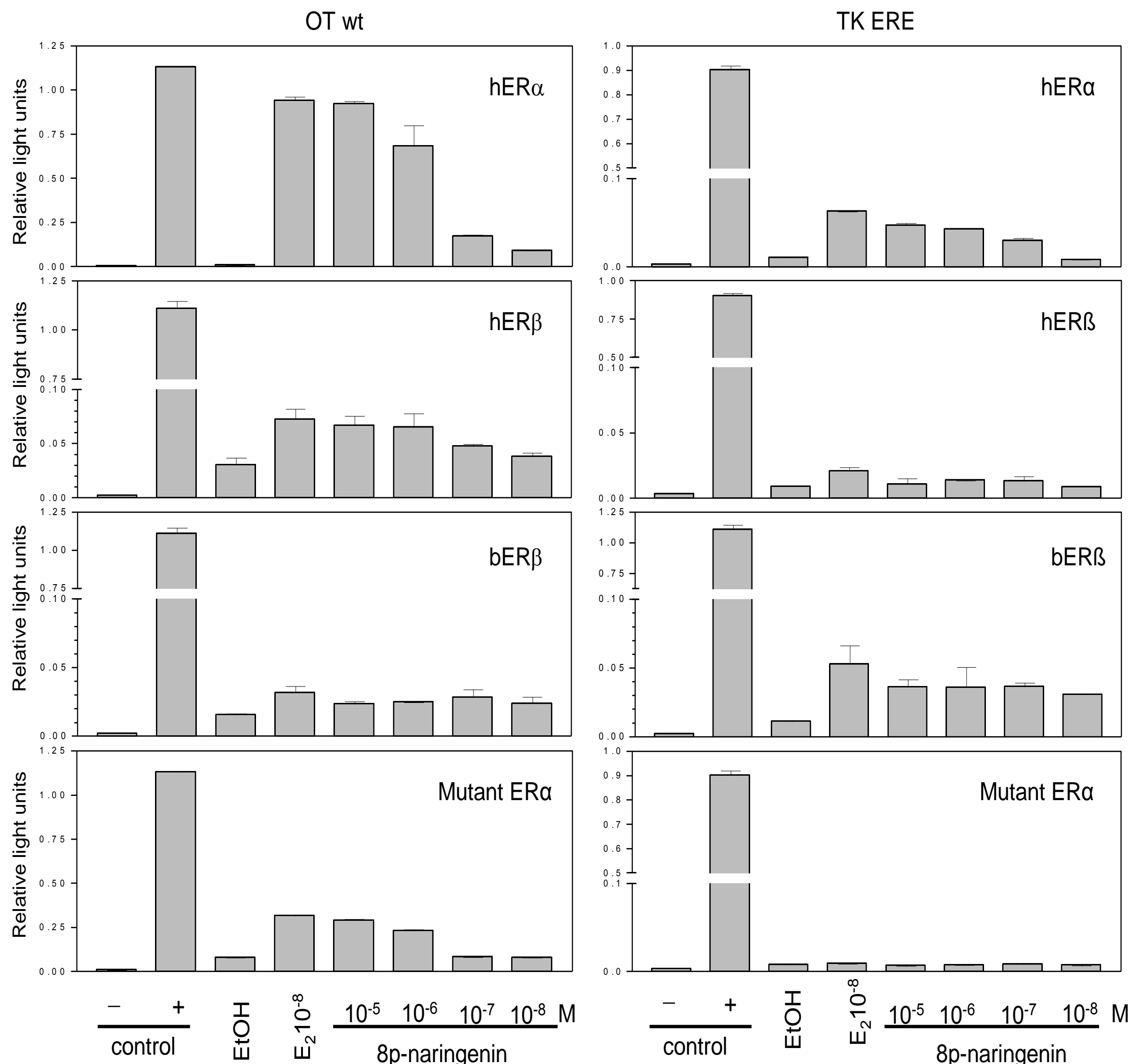

Figure 2. Transcriptional activity of 8p-naringenin on the oxytocin promoter and the thymidine kinase-ERE promoter by different subtype of estrogen receptors in MDA-MB 231cells. MDA-MB 231 cells were transfected with the oxytocin promoter construct (OTwt) or the thymidine kinase-ERE promoter construct (TK ERE) and human estrogen receptor α (hERα), human estrogen receptor ß (hERß), bovine estrogen receptor ß (bERß) or estrogen receptor α mutant unable to bind DNA (mutant ERα) and treated with different concentrations of 8p-naringenin.

Results
Transcriptional activity of phytoestrogens (naringenin, 8p-naringenin and 6DMA-naringenin) on the oxytocin or the thymidine kinase ERE (TK ERE) promoter was summarized in Table 1. The results clearly show that naringenin in the presence of

estrogen receptor α has no major agonistic effect at the concentrations used, either on the oxytocin or on the thymidine kinase-ERE promoter. Only an extremely weak agonistic activity on the oxytocin promoter could be detected at 10^{-5} M. In the presence of human estrogen receptor ß or bovine estrogen receptor ß, naringenin shows agonistic activity with half-maximal concentrations around 10^{-6} M.

Table 1. Transcriptional activity of phytoestrogens (naringenin, 8p-naringenin and 6DMA-naringenin) on the oxytocin or the thymidine kinase ERE (TK ERE) promoter. (*: low stimulation at high concentration of naringenin, **: not dose-dependent)

Promoter	Oxytocin				TK ERE			
Receptors / Compounds	hERα	hERβ	bERβ	Mutant hERα	hER α	hERβ	bERβ	Mutant hERα
Naringenin	-*	+	+	-	-	+	+	-
8p- Naringenin	+	+**	-	+	+	-	+	-
6DMA- Naringenin	+	-	-	+	+	-	-	-

In the presence of the estrogen receptor α mutant unable to bind DNA, the lack of significant effects of naringenin corresponds to the results obtained with the wild type estrogen receptor α (Figure 1). In parallel experiments using 8p-naringenin which is found in hops, the MDA-MB 231 cells were transfected as described above with the thymidine kinase-ERE-promoter and co-transfected with the different subtypes of estrogen receptors as described above. The transfected cells were treated with increasing concentrations of 8p-naringenin (Figure 2). The results clearly show that 8p-naringenin has a high agonistic effect on the oxytocin promoter and the thymidine kinase-ERE promoter in the presence of human estrogen receptor α. Half-maximal stimulation was obtained at 10^{-7} M to 10^{-6} M on the oxytocin promoter, in contrast to 10^{-8} M to 10^{-7} M on the thymidine kinase-ERE promoter. In the presence of human estrogen receptor β, 8p-naringenin has as agonistic effect on the oxytocin promoter, in the presence of bovine estrogen receptor β, 8p-naringenin did not show any dose-dependent agonistic effect. The transcriptional stimulation of the thymidine kinase–ERE promoter observed in the presence of bovine estrogen receptor ß is not dependent on the dose of 8p-naringenin and could be also observed at concentration as low as 10^{-8} M.

Apparently, there are significant functional differences between human and bovine estrogen receptor β. In order to discriminate between classical and non-classical estrogenic effects, the estrogen receptor α mutant unable to bind to DNA was used. The promoter-specific selective agonistic action clearly shows that on the oxytocin promoter, ligand-activated estrogen receptor α does not need to bind to DNA to stimulate the transcriptional activity. In the other experiment following the same protocol, the effects of the other substituted naringenin, (6DMA-naringenin), was investigated (Figure 3).

Discussion

In this study, the transcriptional effect of xenoestrogens was measured in the MDA-MB 231 transfection system. One of the most important of them is phytoestrogens, which are synthesized by plants. Environmental estrogens exert their effects through classical, genomic, or nongenomic pathways. Due to their similarity with the endogenous hormones, these compounds can bind to nuclear receptors. Their affinities for ERα and ERβ are relatively weak compared to endogenous E2. Thus, they can have agonist or antagonist activity depending on the presence of E2 (13). It has been proved that some isoflavones are selective estrogen receptor modulators that have higher affinity to ERβ than ERα (2, 14).

Environmental estrogens have much lower (up to 100 fold) affinity for nuclear receptors compared to the endogenous ligands (E2). Thus, even low concentrations of environmental estrogens can trigger an altered response of the biological systems. This interference is often achieved by the activation of non-genomic pathways. There are numerous non genomic pathways affected by isoflavones, such as non-genomic signaling mediated by oxidative stress pathways, tyrosine kinases, nuclear factor kappa B, and extracellular-signal-regulated kinases (15, 16). In addition to classical ERs, isoflavones serve as ligands for peroxisome-proliferator-activated receptors, the nonclassical estrogen receptor GPER1, the estrogen-related receptors, and the aryl hydrocarbon receptor (15, 17, 18, 19).

In addition to these direct actions to modulate signaling pathways, isoflavones can change epigenetic marks by altering activities of DNA and histone methyltransferases, NAD-dependent histone deacetylases, and other modifiers of chromatin structure (20). In the present study here, the transcriptional effects of phytoestrogens were

investigated. This study focused on the effects of naringenin, 6-(1, 1-dimethylallyl) naringenin (6DMA-naringenin) and 8-prenylnaringenin (8p-naringenin), as these compounds have been shown to exhibit considerable selectivity for one of the two subtypes of estrogen receptors (21, 22).

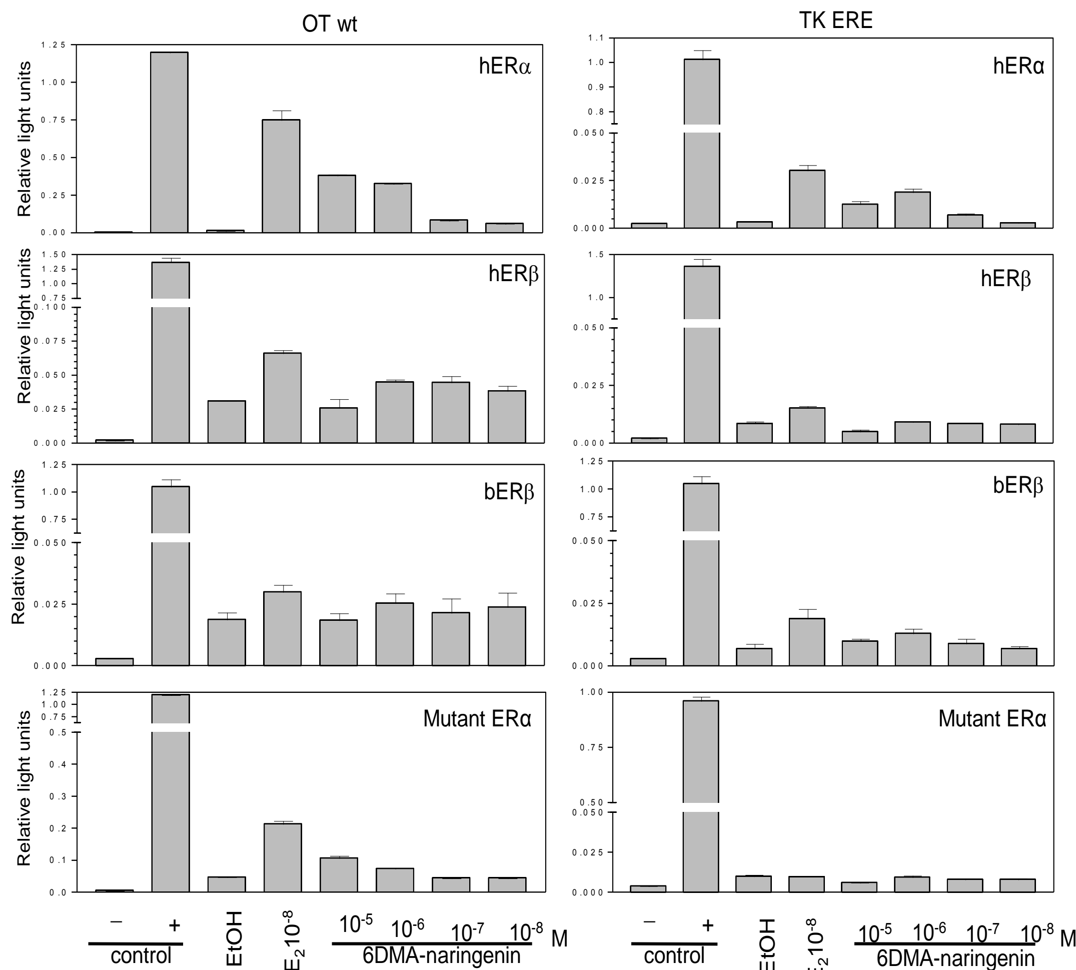

Figure 3. Transcriptional activity of 6DMA-naringenin on the oxytocin promoter and the thymidine kinase-ERE promoter by different subtype of estrogen receptors in MDA-MB 231cells. MDA-MB 231 cells were transfected with the oxytocin promoter construct (OTwt) or the thymidine kinase-ERE promoter construct (TK ERE) and human estrogen receptor α (hERα), human estrogen receptor ß (hERß), bovine estrogen receptor ß (bERß) or estrogen receptor α mutant unable to bind DNA (mutant ERα) and treated with different concentrations of 6DMA-naringenin.

In the previous study, we revealed that some metal ions show estrogenic activity by classical or non-classical mechanisms as well as some metal ions exhibit estrogenic activity by undetermined mechanisms in transfected MDA-MB 231 cell line (23). Phytoestrogens, Gen, Res and Phl, which are structurally or functionally similar to E2, and can bind to estrogen receptors selectively. Hence, phytoestrogens reveal variable degrees of estrogenic and anti-estrogenic activities (22). It has been shown that phytoestrogens such as genistein, equol and daidzein are able to mediate the proliferation of breast cancer cell lines (24, 25). Mueller *et al* performed a comprehensive analysis and potency comparison of phytoestrogens and their human metabolites for ER binding, induction/suppression of ERα and ERβ transactivation and coactivator recruitment in human cells. Results of this study showed the soy-derived isoflavones, coumestrol, resveratrol, and zearalenone would appear to have the potential for effectively functioning as endocrine disruptor (1). In particular, the modulation exerted by isoflavones on cancer cell lines appears dose dependent, with some doses rising and other doses decreasing cell proliferation (26, 27). A previous study using MCF-7 derived cells (21) showed that 8-prenyl-naringenin and 6-(1, 1-dimethylallyl) naringenin at concentrations of 10^{-6} M and 5×10^{-6} M, respectively, stimulate transactivation to the same amount as estradiol at 10^{-8} M, whereas simple naringenin shows only very weak estrogenic activity. The results of the present study clearly demonstrate

the subtype-selective activation of estrogen receptor α and β by the phytoestrogen naringenin (activating estrogen receptor β) and its substituted forms 8-prenyl-naringenin and 6-(1, 1-dimethylallyl) naringenin (activating estrogen receptor α), on the ERE-controlled promoter as well as on the oxytocin gene promoter. However, in contrast to the activation of the ERE-controlled promoter, transcriptional up-regulation of the oxytocin gene promoter by the substituted naringenins bound to estrogen receptor α was not dependent on binding of the activated estrogen receptor to the promoter, as shown by use of a mutant estrogen receptor α unable to bind to DNA. Recently investigation assesses modulation of phytoestrogens on transcriptional activity of ERRγ. Findings of this study showed that the phytoestrogen equol acts as an ERRγ agonist (8). All phytoestrogens used in this study act as agonists, showing at least a partial selectivity for one of the two estrogen receptor subtypes. In conclusion, this selectivity presumably is the basis for the specific effects of these alimentary estrogenic substances that can be used for treatment of diseases or the improvement of reproductive health.

References

1. Mueller SO, Simon S, Chae K, Metzler M, Korach KS. Phytoestrogens and Their Human Metabolites Show Distinct Agonistic and Antagonistic Properties on Estrogen Receptor α (ERα) and ERβ in Human Cells. Toxicol Sci. 2004; 80, 14-25. PMID: 15084758

2. Branham WS, Dial SL, Moland CL, et al. Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. J Nutr. 2002; 132, 658-64. PMID: 11925457

3. Setchell KD. Phytoestrogens: the biochemistry, physiology and implications for human health of soy isoflavones. Am J Clin Nutr. 1998; 68(6):1333-146. PMID: 9848496

4. Cornwell T, Cohick W, Raskin I. Dietary phytoestrogens and health. Phytochemistry. 2004; 65: 995-16. PMID: 15110680

5. Price KR, Fenwick GR. Naturally occurring oestrogens in foods- a review. Food Addit Contam. 1985; 2(2):73-106. PMID: 4018320

6. Kurzer MS. Supplement: dietary supplement use in women: current status and future directions phytoestrogen supplement use by women. J. Nutr. 2003; 133: 1983-86. PMID: 12771350

7. Klinge K. Estrogen receptor interaction with estrogen response elements. Nucleic Acid Res. 2001; 29: 2905-19. PMID: 11452016

8. Satih S, Chalabi N, Bignon YJ, Rabiau NG, Bosviel RM, Bernard-Gallon DJ, et al. Gene Expression Profiling of Breast Cancer Cell Lines in Response to Soy Isoflavones Using a Pangenomic Microarray Approach. OMICS. 2010; 14(3): 231-8. PMID: 20455703

9. Hirvonena J, Rajalina AM, Wohlfahrt G, Adlercreutzc H, Wähälä K, Aarnisaloa P. Transcriptional activity of estrogen-related receptor (ERR) is stimulated by the phytoestrogen equol. J Steroid Biochem Mol Biol. 2011; 123(1-2):46-57. PMID: 21056663

10. Koohi MK, Ivell R, Walther N. Transcriptional activation of the oxytocin promoter by oestrogens uses a novel non-classical mechanism of oestrogen receptor action. J Neuroendocrinol. 2005; 17(4): 197-207. PMID: 15842231

11. Stedronsky K, Telgmann R, Tillmann G, Walther N, Ivell R. The affinity and activity of the multiple hormone response element in the proximal promoter of the human oxytocin gene. J Neuroendocrinol. 2002; 14(6): 472-85. PMID: 12047722

12. Walther N, Lioutas C, Tillmann G, Ivell R. Cloning of bovine estrogen receptor beta (ERbeta): expression of novel deleted isoforms in reproductive tissues. Mol Cell Endocrinol. 1999; 152(1-2): 37-45. PMID: 10432221

13. Shanle EK, Xu W. Endocrine disrupting chemicals targeting estrogen receptor signaling: identification and mechanisms of action. Chem Res Toxicol. 2011; 24(1): 6-19. PMID: 21053929

14. Lóránd T, Vigh E, Garai J. Hormonal action of plant derived and anthropogenic non-steroidal estrogenic compounds: phytoestrogens and xenoestrogens. Curr Med Chem. 2010; 17(30) 3542-74. PMID: 20738246

15. De Souza PL, Russell PJ, Kearsley JH, Howes LG. Clinical pharmacology of isoflavones and its relevance for potential prevention of prostate cancer. Nutr Rev. 2010; 68(9): 542-55. PMID: 20796219

16. Watson CS, Alyea RA, Jeng YJ, Kochukov MY. Nongenomic actions of low concentration estrogens and xenoestrogens onmultiple tissues. Mol Cell Endocrinol. 2007; 274(1-2): 1-7. PMID: 17601655

17. Dang ZC. Dose-dependent effects of soy phyto-oestrogen genistein on adipocytes: mechanisms of action: other review. Obes Rev. 2009; 10(3): 342-9. PMID: 19207876

18. Prossnitz ER, Barton M. Signaling, physiological functions and clinical relevance of the G protein-coupled estrogen receptor GPE. Prostaglandins Other Lipid Mediat. 2009; 89(3-4): 89-97. PMID: 19442754

19. Suetsugi M, Su L, Karlsberg K, Yuan YC, Chen S. Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptor. Mol Cancer Res. 2003; 1(13): 981-91. PMID: 14638870

20. Labinsky N, Csiszar A, Veress G, et al. Vascular dysfunction in aging: potential effects of resveratrol, an anti-inflammatory phytoestrogen. Curr Med Chem. 2006; 13(9): 989-96. PMID: 16611080

21. Zierau O, Gester S, Schwab P, Mety P, Kolba S, Wulf M, et al. Estrogenic activity of the phytoestrogens naringenin, 6-(1, 1-dimethylallyl) naringenin and 8-prenylnaringenin. Planta Med. 2002; 68(5): 449-51. PMID: 12058324

22. Vollmer G, Zierau O. What are phytoestrogens and phyto-SERMS. pharm Unsere Zeit. 2004; 33(5): 378-83. PMID: 15497333

23. Koohi MK, Zayerzadeh E, Eslami M, Zadeh Hashem E. Transcriptional effects of metal ions on the bovine oxytocin and the thymidine kinase-ERE promoter through the estrogen receptor α in MDA-MB 231 breast cancer cell line. IJVM. 2012; 6:79-87.

24. De la Parra C, Otero-Franqui E, Martinez-Montemayor M, Dharmawardhane S. The soy isoflavone equol may increase cancer malignancy via up-regulation of eukaryotic protein synthesis initiation factor eIF4G. J Biol Chem. 2012; 287(50):41640-50. PMID: 23095751

25. Carreau C, Flouriot G, Bennetau-Pelissero C, Potier M. Respective contribution exerted by AF-1 and AF-2 transactivation functions in estrogen receptor α induced transcriptional activity by isoflavones and equol: consequence on breast cancer cell proliferation. Mol Nutr Food Res. 2009; 53(5): 652-8. PMID: 19065587

26. Mousavi Y, Adlercreutz H. Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. Steroids. 1993; 58(7): 301-4. PMID: 8212077

27. Sampey BP, Lewis TD, Barbier CS, Makowski L, Kaufman DG. Genistein effects on stromal cells determines epithelial proliferation in endometrial co-cultures. Exp Mol Pathol. 2011; 90(3): 257-63. PMID: 21281625