Glycitein induces cellular differentiation in nontumorigenic prostate epithelial cells

Elizabeth A. Clubbs and Joshua A. Bomser

OSU Interdisciplinary PhD program in Nutrition, The Ohio State University, Columbus OH 43210, USA

ABSTRACT

Epidemiological and experimental evidence suggests that increased consumption of soy is associated with a reduced risk for prostate cancer. Soy isoflavones are thought to be responsible, in part, for this anticancer activity. Soy isoflavones have been shown to induce cellular differentiation in a number of tissues. However, isoflavone-induced differentiation in the prostate has not been examined. The present study examined the effects of the soy isoflavone, glycitein, on luminal and basal cell differentiation in a nontumorigenic prostate epithelial cell line (RWPE-1). Differentiation was characterized by inhibition of cellular proliferation, cell cycle arrest, and cytokeratin expression. Cytokeratins are differentially expressed among epithelial cell types with luminal prostate epithelial cells expressing cytokeratins 8/18 while basal epithelial cells express cytokeratins 5/14. Treatment of RWPE-1 cells with the soy isoflavones genistein, daidzein, equol, and glycitein (0-50μM) significantly inhibited cellular proliferation at 50μM and glycitein inhibited cellular proliferation at 5 and 50μM. Expression cytokeratin 18 was increased upon treatment of RWPE-1 cells with with N-(4-hydroxyphenyl) retinamide (4-HPR, 1μM), while glycitein treatment (50μM, 8 d) significantly reduced expression of this luminal marker. These data suggest that glycitein may induce basal cell differentiation in the RWPE-1 cell line. Maintaining the basal cell population within the prostate may represent a novel mechanism by which soy isoflavones reduce prostate cancer risk.

INTRODUCTION

- Prostate cancer (PCa) is the third leading cause of cancer related deaths among American males.
- Prostate carcinogenesis is characterized as a continuum of impairment of the homeostatic control governing differentiation, proliferation, and apoptosis of the prostate epithelium.
- The prostate epithelium consists of 2 primary differentiated cell types, luminal and basal and are characterized primarily by their unique cytokeratin profiles.
- Loss of luminal cell differentiation and a concomitant increase in the proliferation is initially observed in low grade prostatic intraepithelial neoplasia (PIN).
- Progression to high grade PIN involves disruption and partial loss of the basal cell population with a complete loss of this cell population observed in PCa.
- Asian populations have lower PCa incidence and mortality but similar rates of incidence of low grade PIN when compared to the United States. The reduced incidence of PCa is attributed to increased consumption of soy and soy-containing products.
- This suggests that soy consumption may reduce PCa incidence by maintaining the basal cell population in the prostate epithelium; however, this hypothesis is yet to be tested.

The objective of this study was to identify a potential mechanism of preserving the basal cell population during PCa progression via isoflavone-induced basal cell differentiation of an intermediate cell population.

HYPOTHESIS

We hypothesize that soy isoflavones may reduce prostate cancer risk by increasing prostate epithelial cell differentiation.

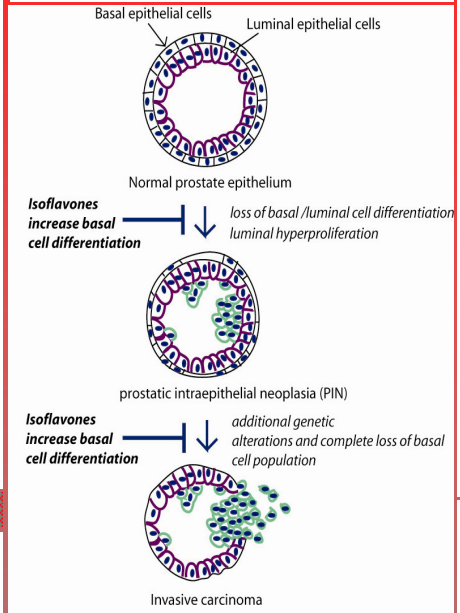

Figure 1. Progression of prostate cancer. Loss of basal and luminal cell differentiation is accompanied by hyperproliferation within the luminal compartment of the prostate. Further genetic alterations and complete loss of the basal epithelium is characteristic of invasive prostate

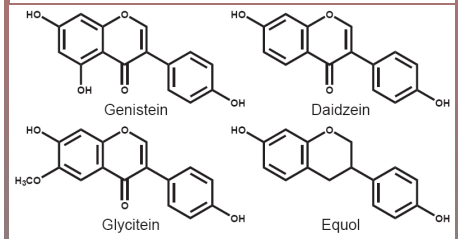

Figure 2. Chemical structures of the major (genistein and daidzein), minor (glycitein) soy isoflavones and the daidzein metabolite (equol).

MATERIALS & METHODS

To test our hypothesis, the effects soy isoflavones on prostate epithelial proliferation, cell cycle distribution and cell differentiation were measured using the 3-[4,5-dimethylthiazol-2-yl]-2-,5-diphenyltetrazolium bromide (MTT) assay, flow cytometric analysis, and western analysis, respectively.

Statistical significance between groups was determined by one-way analysis of variance with Tukey's post-hoc comparisons. Values of $p<0.05$ were considered significant.

RESULTS and DISCUSSION

Question 1: Do soy isoflavones reduce the proliferation of nontumorigenic prostate epithelial cells?

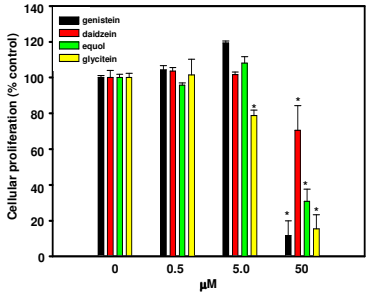

Figure 3. RWPE-1 cells were treated with 0-50μM genistein, daidzein, equol, or glycitein for 8 days. Cellular proliferation was measured using the MTT assay. Data are given as means ± R.S.E.

Conclusion 1: All isoflavones tested reduced RWPE-1 cell proliferation at concentrations of 50μM . Of these, glycitein was the only isoflavone that reduced cellular proliferation at 5μM. Genistein, but not glycitein was cytotoxic at 50μM.

Question 3: Does glycitien induce expression of the luminal cell marker, cytokeratin 18?

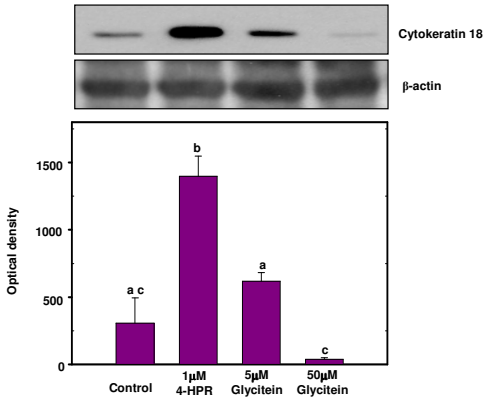

Figure 4. RWPE-1 cells were treated with 1μM 4-HPR, 5 or 50μM glycitein, or vehicle alone for 8 days. 4-HPR, a known inducer of luminal differentiation, was used as a positive control. Cytokeratin expression is given as representative immunoblot and for quantification. Data given as means ± S.E.M. (n=3)

Conclusion 3: Unlike 4-HPR, a known inducer of cytokeratin 18 expression and luminal differentiation, 50μM glycitein decreased cytokeratin 18 expression, showing that glycitien does not induce luminal differentiation.

Question 2: We show that glycitein reduces RWPE-1 cellular proliferation, does glycitein alter cell cycle distribution?

Table 1. Cell cycle analysis of RWPE-1 cells treated 8 days as measured by flow cytometry. 4-HPR is a synthetic retinoid known to induce G_0/G_1 cell cycle arrest and was used as a positive control. Data are given as means ± R.S.E.

Cell Cycle Distribution	Cell Cycle Distribution (%)			
	Control	4-HPR	5μM glycitein	50μM glycitein
G_0/G_1	$63.6^{a} \pm 0.8$	$71.5^{b} \pm 2.7$	$65.9^{a} \pm 1.4$	$62.9^{a} \pm 1.2$
S	$29.2^{a} \pm 2.8$	$12.8^{c} \pm 0.6$	$26.2^{ab} \pm 1.2$	$24.1^{b} \pm 0.9$
G_2/M	$7.2^{a} \pm 1.9$	$15.8^{b} \pm 2.2$	$7.9^{a} \pm 0.2$	$13.0^{b} \pm 0.7$

Conclusion 2: 50μM glycitein was required to reduce S phase and increase G_2/M phase of the cell cycle as compared to the control whereas smaller concentrations of glycitein did not effect cell cycle distribution.

Question 4: Does glycitien induce expression of the basal cell markers, cytokeratin 5 and p63?

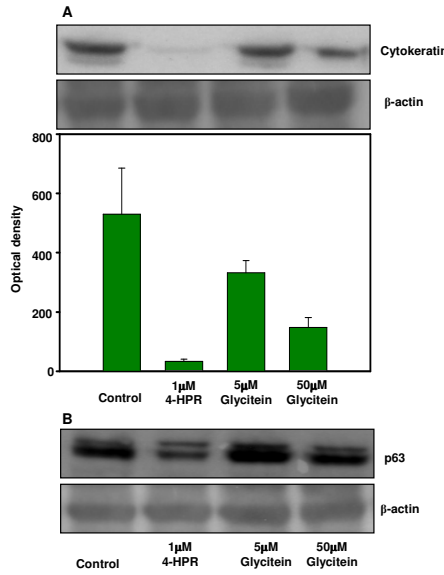

Figure 5. RWPE-1 cells were treated with 1μM 4-HPR, 5 or 50μM glycitein, or vehicle alone for 8 days. 4-HPR, a known inducer of luminal differentiation, was used as a negative control. Cytokeratin expression is given as representative immunoblot and for quantification. Data given as means ± S.E.M. (n=3)

Conclusion 4: Glycitein maintained cytokeratin 5 expression and increased expression of p63 suggesting glycitein induces basal cell differentiation in the RWPE-1 cell line. The negative control, 4-HPR, decreased expression of cytokeratin 5 and p63.

CONCLUSIONS

Taken together these data suggest that glycitein induces basal cell differentiation of prostate epithelial cells. Loss of the basal epithelium occurs during progression of precancerous lesions in the prostate to overt prostate cancer. Preserving the basal cell population may reduce the risk of prostate cancer incidence.

ACKNOWLEDGMENTS

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