Isoflavone-induced activation of extracellular signal-regulated kinase (ERK 1/2) in non-tumorigenic prostate epithelial cells

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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. The present study evaluated the ability of four isoflavones (genistein, daidzein, glycitein, and equol) to modulate extracellular signal-regulated kinase (ERK 1/2) activity in a non-tumorigenic prostate epithelial cell line (RWPE-1). ERK 1/2 controls cellular proliferation and differentiation; however, the specific role of ERK 1/2 signaling in prostate carcinogenesis remains unknown. Treatment of cells with genistein, daidzein, equol, and glycitein (10µM) increased ERK 1/2 activity in a time- and concentration-dependent manner. Further characterization of genistein-induced ERK 1/2 activation suggests involvement of a novel vascular endothelial growth factor (VEGF)-ERK1/2 signaling pathway.

INTRODUCTION

Prostate cancer is the second leading cause of cancer-related deaths in American males. 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. Understanding the mechanisms by which the prostate utilizes soy isoflavones to alter cellular processes will allow for future treatment and prevention of prostate cancer. The objective of this study is to determine the mechanisms by which soy isoflavones modulate signal transduction pathways associated with prostate carcinogenesis, specifically, the mitogen-activated protein kinase pathways (MAPK). At present, three MAPK signaling pathways have been characterized that promote carcinogenesis and include the extracellular signal-regulated kinase cascade (ERK 1/2), p38, and c-Jun N-terminal kinase (JNK). Here, we show that isoflavones, particularly glycitein, increases ERK 1/2 activation at physiological concentrations. Classically, isoflavones have been associated with cellular responses via the estrogen receptor (ER). However, we show glycitein activates the ERK 1/2 cascade via a novel signaling pathway.

MATERIALS AND METHODS

The human prostate epithelial cell line (RWPE-1) and PC-3 metastasized prostate cell line were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). RWPE-1 cells were maintained in keratinocyte-serum-free medium (KSF) (GIBCO Laboratories, Grand Island, NY) supplemented with 50µg/ml bovine pituitary extract, 5% L-glutamine and 5ng/ml epidermal growth factor (EGF). PC-3 cells were maintained in RPMI 1640 medium in a humidified incubator (5% CO2, 5% O2, 90% N2) at 37°C. The human umbilical vein endothelial cell (HUVEC) line was obtained from the American Type Culture Collection (ATCC) (Rockville, MD) and obtained from the American Type Culture Collection (ATCC) (Rockville, MD).

DISCUSSION AND CONCLUSIONS

All isoflavones tested activated ERK 1/2 at 10 µM in non-tumorigenic (RWPE-1) but not tumorigenic (PC-3) prostate cells. Of these, glycitein was the most potent inducer of ERK 1/2 activity in the RWPE-1 cell line. Glycitein-induced ERK 1/2 activation is both estrogen and androgen-receptor independent. However, inhibition of VEGF tyrosine kinases blocks glycitein-induced ERK 1/2 activation, suggesting involvement of VEGF signaling.

RESULTS

Figure 1. Isoflavones induce ERK 1/2 activity in non-tumorigenic (RWPE-1) but not in tumorigenic (PC-3) prostate cell lines.

Figure 2. Glycitein-induced ERK 1/2 activation (p-ERK1/2) is mediated by an estrogen (A) and androgen (B) independent mechanism. RWPE-1 cells were treated with 10µM antiestrogen (ICI 182, 780) or antiandrogen (hydroxyflutamide) alone or in combination with VEGF 50ng/ml and EGF 5ng/ml.

Figure 3. Glycitein-induced ERK 1/2 activation (p-ERK1/2) is mediated by VEGFR activity. (A) ERK 1/2 activity was measured following treatment of RWPE-1 cells with glycitein (50µM) alone or in combination with VEGF or EGF inhibitor (10µM). (B) RWPE-1 cells were treated with VEGF (50ng/ml) or EGF (5ng/ml) alone or in combination with VEGFR inhibitor (0.1µM).

Figure 4. Immunocytochemical identification of vascular endothelial growth factor receptor (VEGFR1 and 2) in RWPE-1 cells. Human umbilical vein endothelial cell (HUVEC) line served as positive controls for VEGFR1 and 2.

Figure 5. Glycitein (A) and VEGF(80) (B) induced ERK 1/2 activation (E) at different concentration (left panel) and time (right panel) dependent.

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REFERENCE