PB-1

WISP-2 is a secreted protein and can be a marker of estrogen-exposure in MCF-7 cells

Hidekuni Inadera,[a,b,c] Hong-Yan Dong,[b] Hideaki Yurino,[b] and Kouji Matsushima[b]

a) Environmental Science Center, University of Tokyo

b) Department of Molecular Preventive Medicine, Graduate School of Medicine, University of Tokyo

c) Japan Science and Technology Corporation

Introduction

As many structurally diverse chemicals have been reported to function as estrogens, evaluations for estrogenicity of compounds are of widespread concern. Recently, we identified WISP-2 (Wnt-1 inducible signaling pathway protein 2) as a novel estrogen-inducible gene in MCF-7 human breast cancer cells. In this study, we examined whether WISP-2 could be utilized as a marker for screening environmentally relevant compounds for estrogenicity.

Materials and Methods

MCF-7 cells were treated 17- β -estradiol (E2) and various kinds of xenoestrogens (XEs), using diethylstilbestrol (DES), genistein, daidzein, zearalenone, bisphenol-A (BPA) and nonylphenol (NP). After 24h, total RNA was isolated, 20 μ g RNA was separated by gel electrophoresis and performed Northern blot analysis. To characterize WISP-2 protein, we generated polyclonal antiserum directed at a peptide sequence 57-ARRLGEPCDQLHV-69 and 237-CPPSRGRSPQNSAF-250 of human WISP-2. We checked the time course and dose-response induction of WISP-2 protein by E2.

Results

In MCF-7 cells, progesterone, dexamethasone, tri-iodothyronine, and 2,3,7,8-tetracblorodibenzo-p-dioxin did not regulate the expression of WISP-2, indicating that its induction is highly specific for hormones that interact with estrogen receptor. Western blot analysis detected WISP-2 protein induced by E2, not only in the cell lysates but also in the culture supernatant of exposed cells, indicating that WISP-2 was a secreted protein. The induction of WTSP-2 protein by E2 in the culture supernatant was dose-dependent with estimated BC_{50} levels between 10 and 100 pM. Our results demonstrated the capacity to screen environmental compounds for estrogenicity via WISP-2 induction.