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Simultaneous disruption of estrogen receptor and Wnt/β-catenin signaling is involved in methyl amooranin-mediated chemoprevention of mammary gland carcinogenesis in rats

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Abstract Methyl-amoorain (methyl-25-hydroxy-3-oxoolean-12-en-28-oate, AMR-Me), a novel synthetic oleanane triterpenoid, exerts a striking chemopreventive effect against 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced rat mammary tumorigenesis through antiproliferative and proapoptotic actions. Nevertheless, the underlying mechanisms of action remain to be established. As estrogen receptor (ER) and canonical Wnt/β-catenin signaling are involved in the development and progression of breast cancer, the current study was designed to investigate the effects of AMR-Me treatment on the expressions of ER-α, ER-β, β-catenin and cyclin D1 in rat mammary tumors induced by DMBA. Mammary tumor samples were harvested from an 18-week chemopreventive study in which AMR-Me (0.8–1.6 mg/kg) was shown to inhibit mammary carcinogenesis in a dose–response manner. The expressions of ER-α, ER-β, β-catenin, and cyclin D1 were determined by immunohistochemistry and reverse transcription-polymerase chain reaction. AMR-Me downregulated the expression of intratumor ER-α and ER-β and lowered the ratio of ER-α to ER-β. AMR-Me also reduced the expression, cytoplasmic accumulation, and nuclear translocation of β-catenin, the essential transcriptional cofactor for Wnt signaling. Furthermore, AMR-Me modulated the expression of cell growth regulatory gene cyclin D1, which is a downstream target for both ER and Wnt signaling. AMR-Me at 1.6 mg/kg for 18 weeks did not exhibit any hepatotoxicity or renotoxicity. The results of the present study coupled with our previous findings indicate that simultaneous disruption of ER and Wnt/β-catenin signaling possibly contributes to antiproliferative and apoptosis-inducing effects implicated in AMR-Me-mediated chemoprevention of DMBA-induced breast tumorigenesis in rats. Our results also suggest a possible cross-talk between two key regulatory pathways, namely ER and Wnt/β-catenin signaling, involved in mammary carcinogenesis and the value of simultaneously targeting these pathways to achieve breast cancer chemoprevention.

Keywords Mammary carcinogenesis · DMBA · Oleanane triterpenoid · Estrogen receptors · Cyclin D1 · β-Catenin

Introduction

Breast cancer is a leading cause of death among women between the ages of 40 and 55 worldwide with an estimated 1.4 million women diagnosed with this disease annually [1]. About 233,000 new breast cancer cases and approximately 40,000 deaths are estimated to occur in women in the United States in 2013 [2]. Elevated lifetime exposure to endogenous or exogenous estrogen has been recognized as the single most important risk factor in the occurrence of breast cancer [3]. The principal mechanism of estrogen action in breast tissue is mediated through binding to nuclear estrogen receptors (ERs) [4]. Two isoforms of ER, namely ER-α and ER-β, have been identified. ERs represent a family of proteins that function as ligand-activated transcription factors that bind to promoter regions of ER-regulated genes. The development of healthy mammary glands requires estrogen

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