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ORIGINAL ARTICLE

Gene expression changes and promoter methylation with the combined effects of estradiol and leptin in uterine tissue of the ovariectomized mice model of menopause

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Abstract

Substantial epidemiological studies have shown an association of obesity with the common gynecological malignancy, endometrial cancer. The relevant interactions and contribution of estradiol and the adipose cytokine, leptin, in endometrial lesions are not completely understood. Suitable animal models to understand the physiological response of uterine tissue to the combined effects of estradiol–leptin are lacking. To investigate the effect of estradiol–leptin crosstalk on gene expression and associated altered pathways, we established an ovariectomized mouse model, treated with 17-β estradiol (0.1 µg/mouse subcutaenously., for every 12 h) and/or recombinant mouse leptin (1 µg/g Bwt intraperitoneally., for every 12 h) for 4 h, 20 h, and 40 h. Gene expressions by semi-quantitative RT-PCR, uterine tissue protein phosphorylation status by western blotting and promoter methylation were analyzed in estradiol, progesterone insufficient animals. Semi-quantitative RT-PCR demonstrated significantly increased expression of *Esr*, *Igf1*, *Igfbp3*, *Vegfr1*, and *Vegf*, and significantly decreased expression of *Mmp9* after co-treatment with estradiol and leptin, indicating a common transcriptional network regulated by the treatments. Ovariectomy-induced histomorphological changes were only reversed by estradiol. Methylation-specific PCR, analyzing methylation of CpG sites of *Vegfa*, *Pgr*, and *Igf1*, revealed that transcriptional regulation after hormonal treatments is independent of methylation at the examined CpG sites. Western blot confirmed the increased expression of PSTAT-3 (Ser-727) and PERK1/2 proteins after estradiol + leptin treatment, confirming the estradiol + leptin cross-talk hypothesis. In conclusion, our in vivo studies determined specific gene expression and signaling protein changes, and further unraveled the molecular targets of estradiol + leptin that may perturb endometrial homeostasis and lead to endometrial hyperplasia development in the chronic stimulated state.

Keywords Uterine tissue · Endocrine physiology · Hormone response

Introduction

Endometrial cancer is the fourth most common cancer among women in the developed world [1]. The American Cancer Society has estimated 63,230 new diagnoses of and 11,350 deaths from endometrial cancer in the United States (US) alone in the year 2018. In India, a study conducted in 2013 had shown the existence of 0.88 million cases of endometrial cancer, with an incidence rate of 105.5 per 100,000 women [2]. Risk of this estradiol-dependent gynecological malignancy is associated with obesity, as confirmed by many epidemiological studies till date [3]. Three candidate biological mediators, proposed for obesity-linked endometrial cancer occurrence include the elevated levels of sex steroids, adipokines, and insulin-like growth factors (IGFs). The increased risk of developing postmenopausal breast cancer,

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