INTRODUCTION

Genomic and non-genomic estrogen signaling involves expression of a wide array of receptors that bind to estrogen. Traditionally, estrogen receptors alpha (ERα) and ER-beta (ERβ) are localized to the cytosol and upon binding to estrogen, translocate to the nucleus and function as transcription factors. Several studies have shown that estrogen can activate rapid non-genomic effects via human ERα (hERα) variants located in the plasma membrane. These effects include increases in calcium, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) signaling. The alternative splicing variants of hERα (hERα66) are hERα46 and hERα36 and are expressed via transcription from different promoters than hERα66. Both splice variants retain the DNA-binding domain of hERα66, as well as the ligand binding domains and partial dimerization; however, hERα36 does not retain the transcriptional activation domains (AF-1 and AF-2) of hERα66, while hERα46 lacks AF-1 only.

We previously showed that the rapid estrogen-dependent activation of PKC in ERα-positive growth plate chondrocytes, human articular chondrocytes, and MCF7 breast cancer cells, as well as in ERα-null HCC38 breast cancer cells is membrane-mediated. PKC activation initiates a signaling cascade that results in activation of the ERK1/2 family of MAPKs, providing an alternate method for estrogen to modulate gene expression other than by traditional steroid hormone receptor-mediated pathways. The membrane action of 17β-estradiol (E2) is supported by studies using E2 conjugated to bovine serum albumin (E2-BSA), which cannot pass through the plasma membrane and reach the nuclear receptor, but elicits many of the same effects as E2 including PKC activation. E2 and E2-BSA rapidly increase PKC specific activity if there is a relative difference in the expression of the various estrogen receptors in male versus female human osteoblasts to determine if there is a relative difference in the expression of the various estrogen receptor alpha variants.

METHODS

Total RNA was harvested from confluent cultures of male and female human osteoblast-like cells (MG63 and SAOS2, respectively). Primers sets were designed to determine total ERα expression (hERα66, hERα46, and hERα36), expression of hERα66/hERα46, and hERα36 alone (Fig 1, top panel). RT-PCR was performed with these specific primer sets and the expression was normalized to GAPDH and densitometry analysis was then performed. Flow cytometry of hERα36 was performed in male and female cells using antibodies against the unique C-terminal region of hERα36 to determine protein expression on the membranes.

RESULTS

RT-PCR analysis showed that male cells expressed 55% less total ERα mRNA than female cells. hERα66 plus hERα46 are reduced by 61% and hERα36 is reduced by 41% (Fig 1, bottom panel). These observations were confirmed by flow cytometry, which showed approximately 30% less expression of hERα36 in male cells compared to female cells (Fig 2).

REFERENCES


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