

Estrogen-induced interaction between KLF5 and estrogen receptor (ER) suppresses the function of ER in ER-positive breast cancer cells

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Kruppel-like factor 5 (KLF5) is implicated in human breast cancer by frequent genomic deletion and expressional deregulation, but the molecular mechanisms by which KLF5 affects breast tumorigenesis are still unknown. This study was conducted to examine whether and how KLF5 affects the function of estrogen receptor (ER) in breast cancer cells. Using different cell lines, we found that restored expression of KLF5 inhibited estrogen-promoted cell proliferation in ER-positive MCF-7 and T-47D cell lines but had no effect on ER-negative SK-BR-3 cells. Transcriptional activity of ER was also suppressed by KLF5, as detected by using estrogen-stimulated ER responsive element-mediated reporter assay and expression analysis of ER target genes including *c-MYC* and Cathepsin D (CSTD). Chromatin immunoprecipitation assays showed that KLF5 inhibited ER α binding to the promoter of *c-myc* and *CSTD*. Furthermore, estrogen induced an interaction between KLF5 and ER α . These results suggest that KLF5 inhibits the function of ER α in gene regulation and cell proliferation through protein interaction that interrupts the binding of ER α to target gene promoters to prevent target gene induction.

Human Kruppel-like factor 5 (KLF5/IKLF/BTEB2) has important roles in various aspects of carcinogenesis including cell proliferation, differentiation, cell-cycle regulation and angiogenesis.¹⁻⁸ It belongs to the Sp/KLF zinc finger transcription factor family, which is composed of over 15 mammalian members that share 3 C2H2-type zinc fingers at the carboxyl terminus forming the DNA-binding domain.^{2,9} Interaction between KLF5 and other transcription factors such as CBP/p300,¹⁰ retinoid acid receptor α ⁶ and NF- κ B¹¹ is important in the transcriptional regulatory function of KLF5.

The role of KLF5 in carcinogenesis appears to be context-dependent. KLF5 is a positive regulator of proliferation in untransformed cells^{7,12} and even transforms normal fibroblasts,³ but it suppresses the proliferation of cancer cells from the prostate,⁵ breast⁴ and colon.⁷ Moreover, KLF5 undergoes frequent genomic deletion in human can-

cers,^{4,5} which results in a loss of function for KLF5 during carcinogenesis because KLF5 is haplo-insufficient.⁶ In breast cancer, while the expression of KLF5 is reduced in most cell lines, expression of KLF5 mRNA in primary tumors was reported to correlate significantly with shorter patient survival and increased expression of oncogene HER2 and proliferation marker MKI67.¹³ Although it is clear that KLF5 plays a role in breast cancer, the molecular mechanisms remain to be elucidated.

Approximately 70% of human breast cancers express estrogen receptors (ERs) including ER α and ER β .¹⁴ Many ER α -positive breast cancers require estrogen for cell proliferation, and they undergo apoptotic cell death when deprived of estrogen.¹⁵ Estrogen 17 β -estradiol (E2) plays pivotal roles in normal breast and in the genesis and progression of breast cancer.¹⁶ The biological actions of estrogen are mediated through its binding to ERs, which belong to the nuclear receptor (NR) superfamily of transcription factors. Interestingly, KLF9 (BTEB1), which is highly homologous to KLF5 (BTEB2),⁹ is a negative regulator of ligand-dependent ER α signaling in human Ishikawa endometrial epithelial cells.¹⁷ On the other hand, KLF5 physically interacts with NRs such as retinoic acid receptor α to regulate the transcription of target genes.^{18,19} Taken together, a relationship between KLF5 and ER α could be related to the function of KLF5 in breast cancer.

In this study, we investigated whether and how KLF5 could function as a coregulator of ER α in ER positive breast cancer cells. We demonstrated that KLF5 inhibited ER function in cell proliferation and gene regulation, likely through estrogen-induced protein interaction with ER α .

Key words: KLF5, ER, estrogen, breast cancer

Abbreviations: ChIP: chromatin immunoprecipitation; CSTD: Cathepsin D; ER: estrogen receptor; KLF5: Kruppel like factor 5; NR: nuclear receptors; PCR: polymerase chain reaction; RAR: retinoid acid receptor; siRNA: small interfering RNA

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