siRNA Inhibition of ER-α Expression Reduces KGF-induced Proliferation of Breast Cancer Cells

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Abstract. Background: Keratinocyte growth factor (KGF) produces a rapid increase in the proliferation and motility of estrogen receptor (ER)-positive breast cancer cells which is abolished by estrogen deprivation and/or anti-estrogen treatment. The present study examined the hypothesis that ER-α is involved in the KGF proliferation in MCF-7 cancer cells using small interfering RNA (siRNA) to selectively inhibit ER-α expression. Materials and Methods: At 48 hours following ER-α siRNA transfection, the MCF-7 cells were treated with KGF (50 ng/ml) or vehicle for 24 hours. Cell proliferation was measured using a MTT assay. ER-α protein levels were quantified by Western blotting. Results: ER-α siRNA transfection significantly reduced ER-α expression and MCF-7 cell proliferation. KGF-mediated enhancement of cell proliferation and motile cell morphology were reduced or absent in the siRNA transfected MCF-7 cells. Conclusion: ER-α expression is associated with KGF-induced proliferation of breast cancer cells.

Keratinocyte growth factor (KGF, also designated FGF-7) is a member of the fibroblast growth factor family (1). KGF is produced by stromal tissue and acts at specific KGF receptors (KGFR) found on epithelial cells (2). KGF activates the KGFR to enhance epithelial cell DNA synthesis, proliferation and migration in breast and other tissue (2-4). KGFR is expressed in breast epithelial cells, while KGF is expressed in the stromal cells of breast tissue (5). The mammary glands of adult male and female rats are remarkably sensitive to KGF. Systemic administration of KGF for 3 to 5 days has been found to produce massive mammary ductal hyperplasia and an elevation of mitotic figures (6). Similarly, in female mice with a constitutively up-regulated KGF transgene, mammary epithelial hyperplasia developed and eventually all the animals developed metastatic mammary carcinomas (7). A high level of KGF expression has also been observed in human primary breast tumor specimens and it has been reported that KGF is a paracrine growth factor in breast cancer (8, 9). However, highly malignant, metastatic breast cancer tissue expressed relatively little KGFR (10), suggesting that KGF-mediated stimulation of breast epithelial proliferation and migration may be an early tumorigenic event. Accordingly, we have demonstrated that KGF treatment and/or KGF transfection of MCF-7 and other estrogen receptor (ER)-positive breast cancer cell lines enhanced cell proliferation and motility (11, 12) and up-regulated KGFR gene expression (13), via the MAP kinase pathway (14), while down-regulation of KGFR expression abolished these KGF-mediated effects (15). We also observed that this KGF-induced response in breast cancer cells was abolished either by estrogen deprivation or antiestrogen treatment (11, 16). Therefore, the present study examined the hypothesis that ER-α is involved in the regulation of KGF-mediated stimulation of cancer cells. The hypothesis was tested using small interfering RNA (siRNA) inhibition of ER-α expression in MCF-7 cells.

Materials and Methods

Culture method. The MCF-7, ER-positive breast cancer cells were grown as monolayer cultures as previously described (11). Cancer cell proliferation was measured in ER-α siRNA transfected or sham-transfected MCF-7 cells at 48 hours following KGF treatment.

Transfection method. A 21-23 bp siRNA for ER-α (ER-α siRNA) (New England Biolabs, New England, USA) at a concentration of 100 nM was transfected into MCF-7 cells. One day before transfection, 10⁵ MCF-7 cells were placed into 30 mm culture dishes containing antibiotic-free media. The cells were then transfected with the transfection reagent (Qiagen, Valencia, CA USA) in accordance with the manufacturer’s instructions. The transfection reagent (15 μl) and ER-α siRNA (6 μl) were incubated with the cells in RPMI (serum-free media) for 48 hours. Control cells were incubated in the same volume of transfection reagent without ER-α siRNA. Cellular...
levels of ER-α protein were quantified by Western blotting and normalized to actin as previously described (14).

**Cell proliferation method.** The ER-α siRNA-transfected and the control cells were treated with KGF (50 ng/ml) or vehicle control for 24 hours. At the conclusion of the KGF treatment period, cell proliferation was quantified using the MTT assay (Promega, Madison WI, USA) according to the manufacturer’s instructions.

**Data analysis.** Multiple group comparisons were conducted using ANOVA and Student’s t-test for pair-wise comparisons. Group differences resulting in p-values of less than 0.05 were considered to be statistically significant.

**Results**

Transfection of the MCF-7 cells with the siRNA produced a 64% reduction in expression of ER-α (Figure 1) and a 40% reduction in cell proliferation (p<0.05) (Figure 2). KGF-mediated proliferation in the ER-α siRNA transfected MCF-7 cells was reduced by 54% as compared to the sham-transfected control cells (28% increase vs. 52% increase,
respectively; \(p<0.05\) as shown in Figure 2. KGF-induced changes in cell morphology observed in the sham-transfected control cells (arrow) were absent in the ER-\(\alpha\) siRNA transfected MCF-7 cells (Figure 3).

**Discussion**

It is known that an imperfect estrogen response element (ERE) exists in the flanking region of the KGFR gene and other estrogen‐dependent genes such as \(pS2\) (17). Thus, estrogenic activity may influence the KGF‐induced breast cancer cell motility response by acting as a co‐activator of KGFR and/or other genes involved in the KGF‐mediated proliferation/motility response. The present study indicated that ER-\(\alpha\) is at least partially required for KGF‐induced proliferation of ER‐positive breast cancer cells. A link between ER activation and the mitogenic activity of other growth factors, such as EGF, in breast cancer has also been established. For example, Yarden and coworkers demonstrated that estradiol produced a rapid 3‐fold increase in the expression of EGFR in MCF‐7 cells which was inhibited by antiestrogen pretreatment (18). These investigators reported the presence of an ERE in the promoter region of the EGFR gene and suggested a direct transcriptional mechanism for estrogens in the regulation of EGFR expression.

Since the effect of KGF on cell proliferation was not completely abolished by siRNA silencing of ER-\(\alpha\) expression in the present study, it is possible that non‐receptor‐mediated effects of estradiol, such as enhancement of signal transduction mediators, may be involved in the KGF proliferation effect on cancer cells. Although it appears that ER-\(\alpha\) is primarily responsible for estrogen‐mediated breast cancer cell proliferation, it is possible that ER-\(\beta\) may also be associated with this KGF‐induced effect on cancer cells (19) and this possibility will be further explored.

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**References**


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