Breast cancer cell growth mediated by EGFR/HER1 expression is regulated by p53 suppressor function

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Background and significance

Human epithelial growth factor receptor (EGFR/HER1) is associated with progression of solid tumors. The expression and activity of this growth factor is abnormally elevated in most cancers (1, 2). Our goal is to prevent cell growth in breast cancer by suppression of EGFR gene expression. By preventing cell growth, we hope to stop the spread and improve treatability of breast cancer. A breast suppressor protein, p53, has been implicated in the regulation of EGFR. How p53 affects EGFR transcription remains unknown. We hypothesize that large amounts of p53 available for binding silences EGFR transcription.

Results

EGFR expression in breast cancer cells

- **Fig. 1.** High level of EGFR mRNA in breast cancer cells. A. Total RNA, isolated from normal mammary epithelial cells (MCF-10A) and three different mammary carcinoma cell lines (MCF-7, MDA-MB-231, and MDA-MB-468), was used to measure the level of EGFR mRNA by RT-PCR analysis using mRNA-specific primers in a limited 15 cycles of PCR. RNA samples used in the RT-PCR were derived from: lane 1, yeast RNA; lane 2, MCF-10A; lane 3, MCF-7; lane 4, MDA-MB-231; lane 5, MDA-MB-468. GAPDH mRNA level was measured using a specific primer set, which was used as a loading control. B. qRT-PCR analysis of the EGFR mRNA was accomplished by using EGFR-specific primers. The result represents an average of three separate experiments.

- **Fig. 2.** EGFR promoter activity in breast cancer cells. Human EGFR promoter DNA, containing sequences from nucleotide position -950 to +148, was cloned into a plasmid vector (pBLCAT3) and transfected into four cell lines, as indicated. Expression of CAT (chloramphenicol acetyl transferase) reporter gene in these cells, that is driven by the EGFR promoter, was measured as enzymatic activity of CAT determined by thin layer chromatography. The results show induction of CAT activity relative to the normal breast epithelial cells (MCF-10A). An average of three independent experiments are shown.

- **Fig. 3.** K-RasV12 and SAF-1 induce EGFR promoter function. Breast cancer cells (MDA-MB-468) were transfected with EGFR promoter-containing CAT reporter plasmids, as described in Fig. 2. Some of the cells were co-transfected with either oncogenic K-RasV12 or SAF-1 expression plasmids. Also, some cells were co-transfected with both K-RasV12 and SAF-1 expression plasmids to assess the effect of activated Ras on SAF-1 in the induction of EGFR promoter. Results represent changes in CAT activity relative to the untreated cells. An average of three independent experiments are shown. DNA sequence of the EGFR promoter shows potential SAF-1 binding sites.

- **Fig. 4.** Sequence of promoter region of EGFR. Three potential binding sites for p53 are indicated.

Suppression of EGFR expression

- **Fig. 5.** Abundance of p53 protein and binding to EGFR promoter. Cellular levels of p53 were measured by Western immunoblot analysis, and the data is shown in panel A. Fifty µg protein in nuclear extracts from MCF-10A (lane 1), MCF-7 (lane 2), MDA-MB-231 (lane 3), and MDA-MB-468 (lane 4) cells were fractionated in a 4%/11% SDS-polyacrylamide gel and probed with anti-p53 antibody (Abcam). Migration position of different molecular weight markers are indicated. The membrane was reprobed with human β-Actin which was used as a loading marker. Binding of nuclear proteins to EGFR promoter was assessed by electrophoretic mobility shift assay (EMSA). The panel is shown in panel B. Two DNA fragments containing EGFR promoter sequences from -382 to -270, -269 to -26, and -26 to -140 were radiolabeled with [γ-32P]ATP and incubated in DNA binding reactions to 0.1 µg protein nuclear extracts from MCF-10A (lanes 5 and 6), MCF-7 (lanes 2, 6, and 10), MDA-MB-231 (lanes 3, 7, and 11), and MDA-MB-468 (lanes 4, 8, and 12) cells. The DNA-protein complexes were fractionated in a 6% non-denaturing polyacrylamide gel and autoradiographed. Identity of the DNA-protein complex was determined by using an antibody ablation/supershift assay and the data is shown (lanes 11 and 12). The complex was incubated with 10 µg of different nuclear extracts, as indicated. In some reactions, nuclear extracts were pre-incubated with anti-p53 antibody, as indicated. DNA-protein complexes were resolved in a non-denaturing gel.

Conclusions

These results indicate that loss of p53 binding to EGFR promoter is at least partly responsible for EGFR overexpression in breast cancer cells. Increased binding of p53 or its derivative may reduce EGFR expression. This suggests that EGFR could be targeted directly for breast cancer treatment in the future.

References


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