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 tumor 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

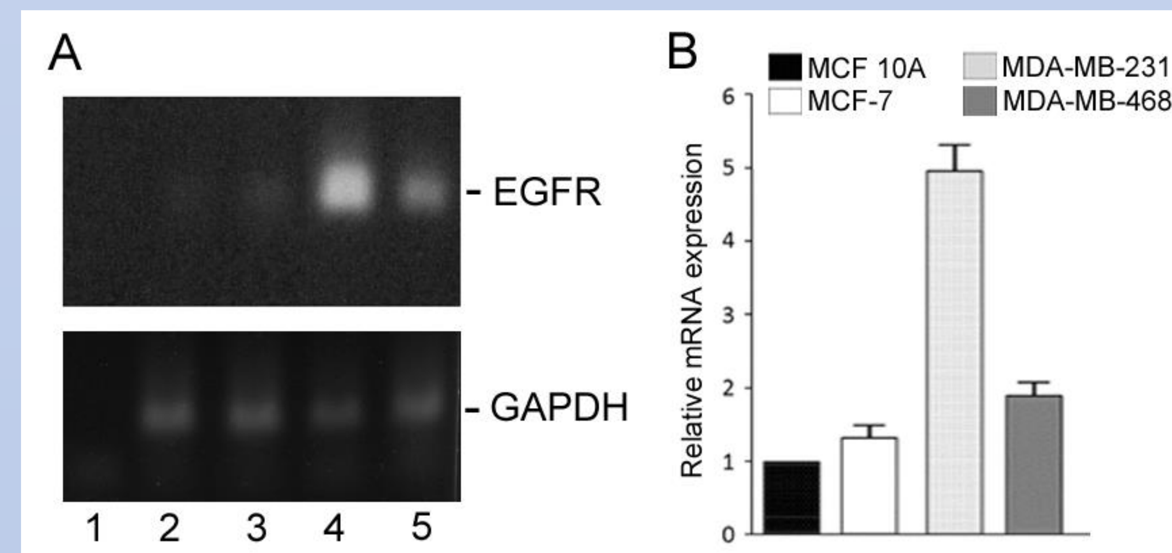

Fig. 1. High level of *EGFR* mRNA in breast cancer cells. A. Total RNA, isolated from normal mammary epithelial cells (MCF-10A) and three different mammary carcinoma cell lines (MCF-7, MDA-MB-231, and MDA-MB-468), was used to measure the level of EGFR mRNA by RT-PCR analysis using mRNA-specific primers in a limited 15 cycles of PCR. RNA samples used in the RT-PCR were derived from: lane 1, yeast tRNA; 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.

High level of EGFR mRNA in breast cancer cells suggests a possible induction of this gene. The inducibility was more profound in MDA-MB-231 cells.

Transcriptional induction of *EGFR* expression in breast cancer cells

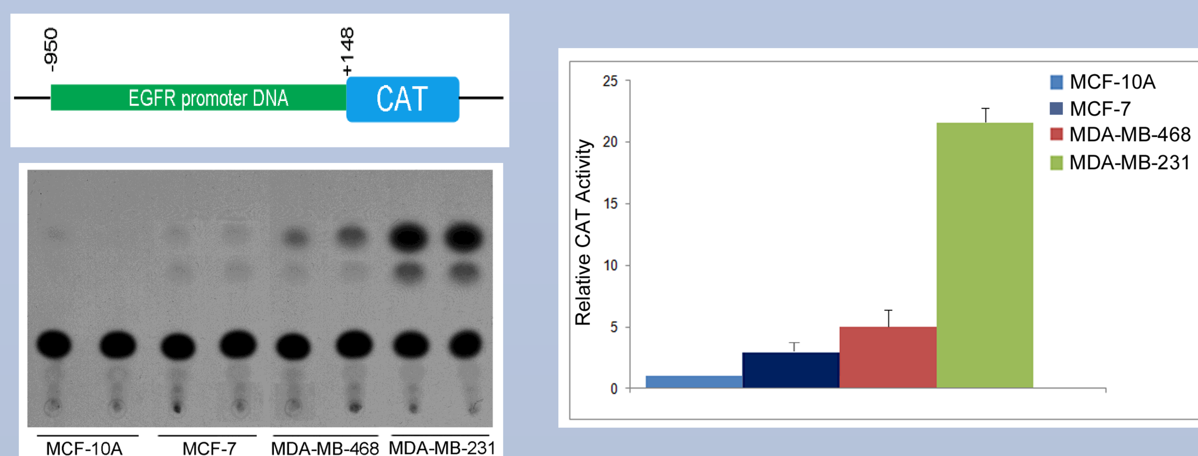

Fig. 2. *EGFR* promoter activity in breast cancer cells. Human EGFR promoter DNA, containing sequences from nucleotide position -950 to +148, was cloned into a plasmid vector (pBLCAT3) and transfected into four cell lines, as indicated. Expression of CAT (chloramphenicol acetyl transferase) reporter 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.

Significant induction of EGFR promoter in MDA-MB-231 cells raises a possibility that transcriptional induction is responsible for increased expression of EGFR.

K-RasV12-SAF1 axis promotes *EGFR* expression

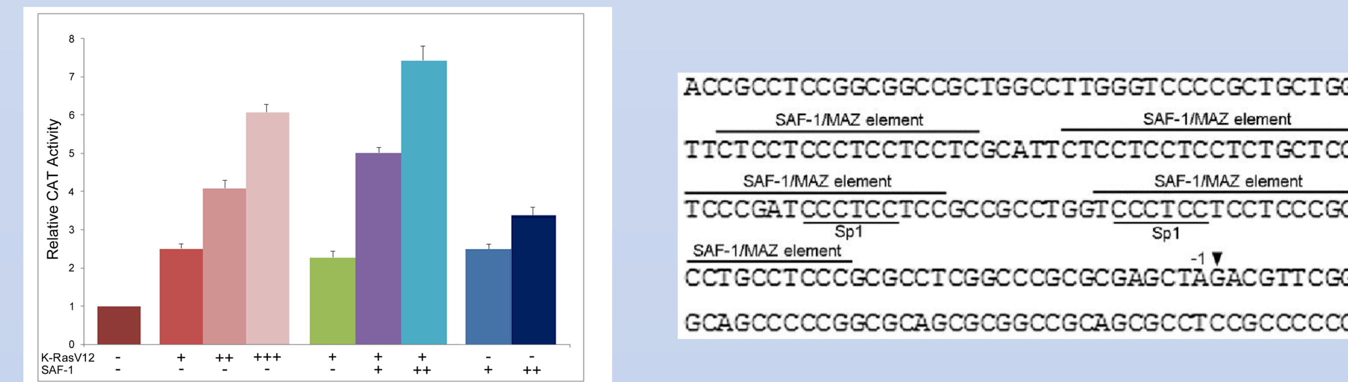

Fig. 3. K-RasV12 and SAF-1 induce *EGFR* promoter function. Breast cancer cells (MDA-MB-468) were transfected with EGFR promoter-containing CAT reporter plasmids, as described in Fig. 2. Some of the cells were co-transfected with either oncogenic K-RasV12 or SAF-1 expression plasmids. Also, some cells were co-transfected with both K-RasV12 and SAF-1 expressing 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.

Presence of a mutant K-Ras in the cells promotes EGFR expression. Furthermore, overexpression of SAF-1 increases EGFR promoter activity with a synergistic role of K-Ras. Together, these findings indicate that interaction of transcription factor SAF-1 to EGFR promoter causes induction of EGFR expression. SAF-1 activity is increased by Ras signaling via MAP kinase pathway (3).

Suppression of *EGFR* expression

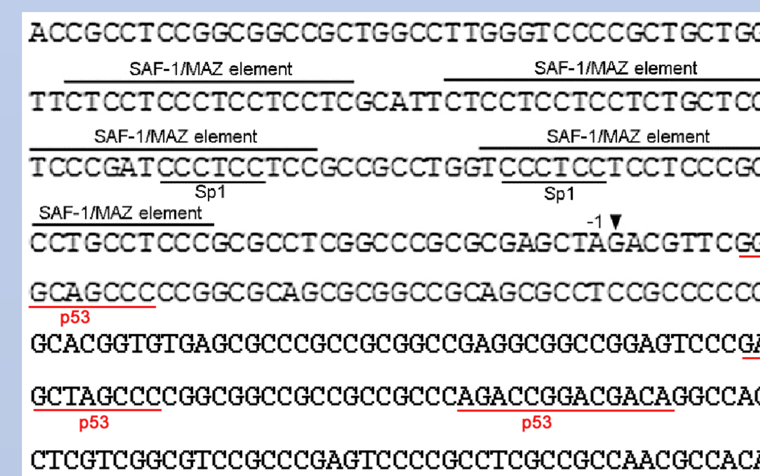

Fig. 4. Sequence of promoter region of *EGFR*. Three potential binding sites for p53 are indicated.

Reduced expression of EGFR in normal breast epithelial cells and some breast cancer cells, such as MCF-7, raises the possibility of a cellular mechanism in these cells that suppresses expression. Analysis of the EGFR promoter (Fig. 4) revealed several binding elements for a transcriptional suppressor, p53 (4), which is implicated in the regulation of EGFR expression in human keratinocytes (5).

p53 level and its interaction with *EGFR* promoter in breast cancer cells

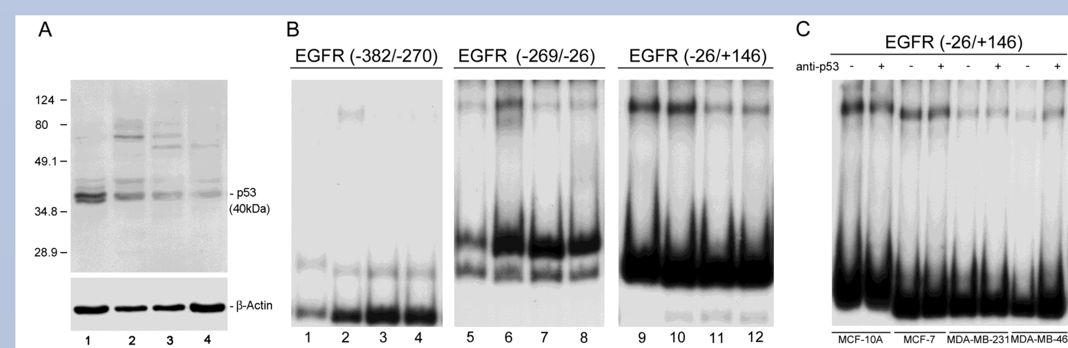

Fig. 5. Abundance of p53 protein and binding to *EGFR* promoter. Cellular levels of p53 were measured by Western immunoblot analysis, and the data is shown in panel A. Fifty μg protein in nuclear extracts from MCF-10A (lane 1), MCF-7 (lane 2), MDA-MB-231 (lane 3), and MDA-MB-468 (lane 4) cells were fractionated 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 re-probed with anti-β-actin, which was used as a loading marker. Binding of nuclear proteins to EGFR promoter was assessed by electrophoretic mobility shift assay, and the data is shown in panel B. Three DNA fragments containing EGFR promoter sequences from -382 to -270, -269 to -26, and -26 to +146 were radiolabeled with ^{32}P-dCTP and used in DNA-binding reactions to 10 μg protein in nuclear extracts from MCF-10A (lanes 1, 5, and 9), 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 in panel C. EGFR DNA (-26/+146) 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.

We have detected multiple p53 proteins in the breast cancer cells while normal breast epithelial cells contain primarily one major isoform with an apparent molecular weight of about 40 kDa. p53 is known to form multiple isoforms due to alternative splicing, and multiple isoforms have been reported in breast cancer cells (6, 7). Shorter isoforms have distinct physiological functions (8). Presence of a shorter isoform at high abundance in normal breast epithelial cells, MCF-10A, and its ability to avidly bind to EGFR promoter suggests that such an interaction of truncated p53 to EGFR promoter may be responsible for suppression of EGFR expression. Absence of a similar truncated shorter version of p53 in breast cancer cells results in less binding to EGFR promoter. This scenario may be at least partly responsible for overexpression of EGFR in breast cancer cells.

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.

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Acknowledgements

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