Background and significance

Breast cancer is the most common cancer in women and has the second highest death rate among cancers in people. Epidermal growth factor receptor (EGFR) family members which include HER1, HER2, HER3, and HER4 perform key roles in determining aggressive growth of breast cancer due to the overexpression of HER1-2. Research shows that in K-Ras-activated SAF1/MAZ-promoted EGFR expression in breast cancer cells. A tumor suppressor protein, p53, and its derivatives suppress SAF-1/MAZ function by binding to the EGFR promoter. Determining how the binding of p53 is affected will introduce new possible targets for breast cancer treatment. We hypothesize that SAF-1/MAZ binding to the EGFR promoter prevents binding of p53 resulting in the increased expression of EGFR and therefore, growth of tumor cells in breast cancer.

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. qRTP-PCR analysis of the EGFR mRNA was accomplished by using a GAPDH 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.

K-Ras-activated SAF1/MAZ promotes EGFR expression

Fig. 2. K-RasV12 and SAF1 induce EGFR promoter function. Breast cancer cells (MDA-MB-468) were transfected with EGFR promoter-containing CAT reporter plasmid. In some assays, cells were co-transfected with either oncogenic K-RasV12 or SAF1 expression plasmids or both 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 SAF-1 binding sites is shown.

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).

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

Fig. 3. Abundance of p53 protein and its 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 [32P]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 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.

We have detected several p53 protein products in the breast cancer cells while normal breast epithelial cells contain primarily one major isoform. p53 is known to form multiple isoforms due to alternative splicing, which have been reported in breast cancer cells (4, 5) with shorter isoforms having distinct physiological functions (6). Presence of p53 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 p53 to EGFR promoter may be responsible for suppression of EGFR expression.

Increased p53 expression in breast cancer cells inhibits EGFR promoter function

Fig. 4. p53 reduces EGFR promoter function. Breast cancer cells (MDA-MB-231) were transfected with EGFR promoter-driven CAT reporter plasmid. In some assays, cells were co-transfected with increasing concentrations (0.5, 1.0 and 5.0 µg) of p53 expression plasmid to evaluate the effect of ectopically expressed p53 in the EGFR expression. Results represent changes in CAT activity relative to the untreated control. In two independent experiments shown, DNA sequence of the SAF-1 binding sites is shown. 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).

Conclusions

Results indicate that tumor suppressor protein p53 and the zinc finger transcription factor SAF-1/MAZ can interact with each other to form a protein-protein complex. SAF-1/MAZ sequences low level of p53 protein in breast cancer cells reducing the binding of p53 to the promoter region on EGFR, consequently resulting in the overexpression of EGFR. Our findings provide a new molecular mechanism that explains the previously reported tumor suppressor function of p53 (7, 8).

References


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