Binding of p53 and SAF-1/MAZ to the *EGFR* promoter controls breast cancer cell growth

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IDEXX BioResearch

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 EGFR (1, 2). Research shows that Ras-activated SAF-1/MAZ promotes *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

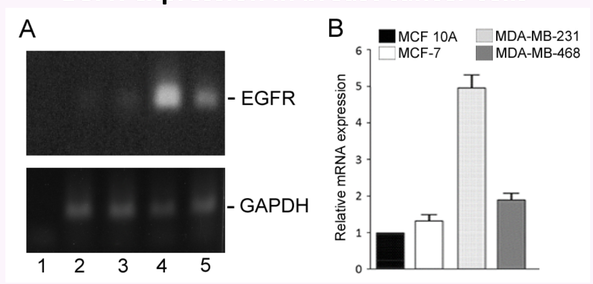

Fig. 1. High level of *EGFR* mRNA in breast cancer cells. A. Total RNA, isolated from normal mammary epithelial cells (MCF-10A) and three different mammary carcinoma cell lines (MCF-7, MDA-MB-231, and MDA-MB-468), was used to measure the level of EGFR mRNA by RT-PCR analysis using mRNA-specific primers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

K-Ras-activated SAF1/MAZ promotes *EGFR* expression

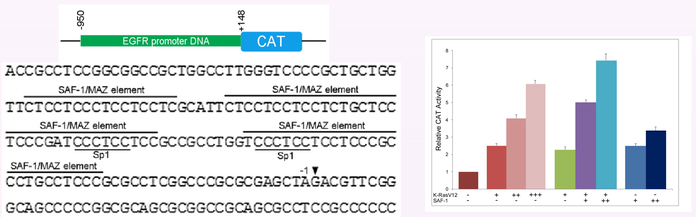

Fig. 2. K-RasV12 and SAF-1 induce *EGFR* promoter function. Breast cancer cells (MDA-MB-468) were transfected with *EGFR* promoter-containing CAT reporter plasmid. In some assays, cells were co-transfected with either oncogenic K-RasV12 or SAF-1 expression plasmids or both to assess the effect of activated 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 *EGFR* promoter shows several 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).

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

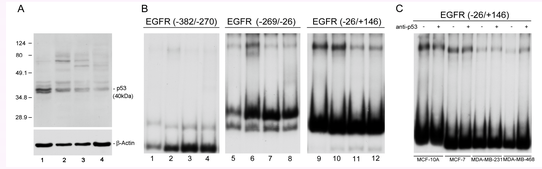

Fig. 3. Abundance of p53 protein and its binding to *EGFR* promoter. Cellular levels of p53 were measured by Western immunoblot analysis, and the data is shown in panel A. Fifty μg protein in nuclear extracts from MCF-10A (lane 1), MCF-7 (lane 2), MDA-MB-231 (lane 3), and MDA-MB-468 (lane 4) cells were 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 ^{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 supershift assay (Panel C). *EGFR* DNA (-26/+146) was incubated with 10 μg of nuclear extracts. 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 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

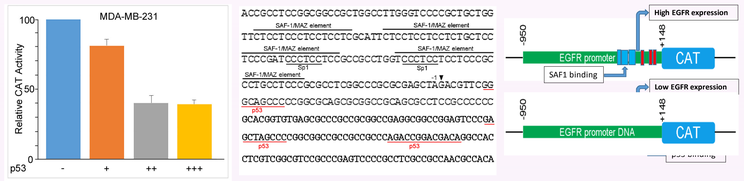

Fig. 4. p53 reduces *EGFR* promoter function. Breast cancer cells (MDA-MB-231) were transfected with *EGFR* promoter-driven CAT reporter plasmid. In some assays, cells were co-transfected with increasing concentrations (0.5, 1.0 and 2.0 μg) of p53 expression plasmid to evaluate the effect of ectopically expressed p53 in the *EGFR* expression. Results represent changes in CAT activity relative to the untreated cells. An average of three independent experiments are shown. DNA sequence of the *EGFR* promoter shows several binding sites for SAF1 and p53.

Results show that ectopic expression of p53 in breast cancer cells reduces CAT reporter gene expression. This suggests that high abundance of p53 and its binding to the EGFR promoter suppresses promoter function and thus reduces expression of EGFR, which is seen in normal breast epithelial cells. In contrast, high level of SAF-1 protein combined with low p53 level in breast cancer cells causes over-expression of EGFR.

Opposing effects of SAF-1 and p53 raises the possibility of mutually exclusive binding of these proteins to EGFR promoter and sequestration of their respective activities by direct interaction among these two proteins.

Interaction of p53 and SAF1/MAZ proteins

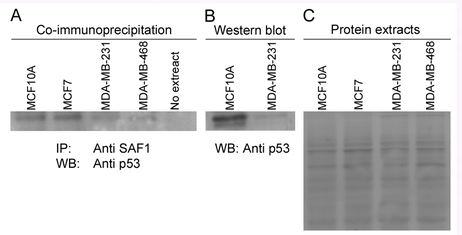

Fig. 5. p53 binds to SAF1/MAZ. Binding of p53 to SAF1/MAZ was examined by co-immunoprecipitation assay and the results are shown in Panel A. One hundred μg protein in nuclear extracts from MCF-10A, MCF-7, MDA-MB-231 and MDA-MB-468 cells were used in the immunoprecipitation (IP) reaction with anti-SAF1 antibody. Immuno-precipitated complexes were recovered by using protein A-agarose. Immuno-complexes were fractionated in a 4%/11% SDS-polyacrylamide gel and probed with anti-p53 antibody in the Western blot (WB) assay. Panel B shows a Western blot analysis of p53 in two nuclear extract preparations, same as those used in panel A. Panel C shows coomassie blue-stained proteins following fractionation of the nuclear extracts in a 4%/11% SDS-polyacrylamide gel, and was used as a loading control.

Results of the Co-IP assay indicate a strong binding of p53 to SAF1/MAZ. Low level of p53 in breast cancer cells (MDA-MB-231 and MDA-MB-468) is efficiently pulled-down by the SAF1/MAZ. This event suggests a potential mechanism of sequestration of p53 in breast cancer cells leading to over-expression of EGFR.

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. Such an interaction sequesters 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 finding provides a new molecular mechanism that explains the previously reported tumor suppressor function of p53 (7, 8).

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Acknowledgements

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