Regulatory mechanism of EGFR/HER1 expression in breast cancer cells involves Ras-mediated activation of SAF-1

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Introduction

Tumor microenvironment (TME) is a dynamic entity which determines tumor growth, invasion and metastasis. A number of cellular processes regulate different elements of the TME and they determine the status of the tumor ranging from dormancy to aggressive growth (1). 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 abnormal expression of these growth factor receptors. Overexpression of EGFR (also known as HER1) in aggressive breast cancer suggests that a cancer cell-specific mechanism could be involved in determining this biosynthetic abnormality. In two-thirds of aggressive breast cancer patients, transcriptional induction of *EGFR* causes high EGFR/HER1 level (2).

Over-expression of HER2 has been emphasized in breast cancer and thus HER2 is regarded as a promising therapeutic target. Interestingly, more than one third of HER2-positive breast tumors also overexpress EGFR/HER1 (3) and these patients who simultaneously overexpress both HER1 and HER2 suffer more from lymph node metastasis in comparison with patients whose tumors overexpress only HER2. Benefits from anti-HER2 targeted therapy (Trustuzumab/Herceptin) is possibly attenuated due to the overexpression of HER1 (4). This finding suggests that HER1 overexpression in the aggressive breast cancer may pose a real problem. Despite many studies aimed at divulging mechanisms to neutralize EGFR/HER1 activity, the root cause of the problem, that is, how it is overexpressed, remains unsolved.

Objective

The goal of this study is to identify the molecular mechanism by which EGFR is over-expressed in breast cancer cells. Since Ras signaling pathway has been implicated in EGFR expression (5) and our recent work indicates that Ras activates SAF1 function (6), we plan to investigate whether EGFR expression involves Ras-SAF-1 pathway.

Results

EGFR expression in breast cancer cells

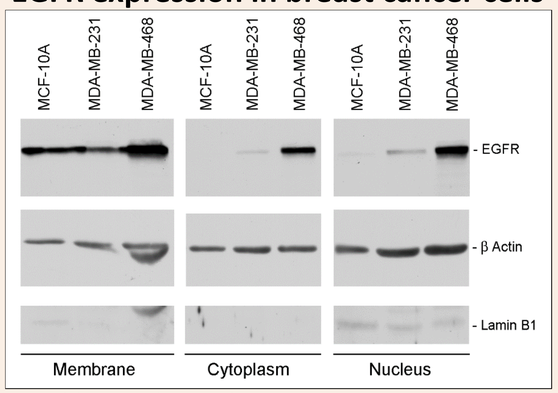

Fig. 1. Western blot analysis of EGFR expression. Proteins in three sub-cellular fractions of human normal mammary epithelial cells (MCF-10A) and two different mammary carcinoma cell lines (MDA-MB-231 and MDA-MB-468) which are derived from human breast cancer patients, were fractionated in SDS-PAGE, transferred to a PVDF membrane and blotted with anti-EGFR antibody (Santa Cruz Biotechnology). The bands were detected after chemiluminescence reaction. The same membrane was re-probed with anti-β Actin and anti-Lamin B1 antibodies, which were used as loading controls.

High level of EGFR in breast cancer cells suggests a possible induction of this gene.

Transcription induction of EGFR expression in breast cancer cells

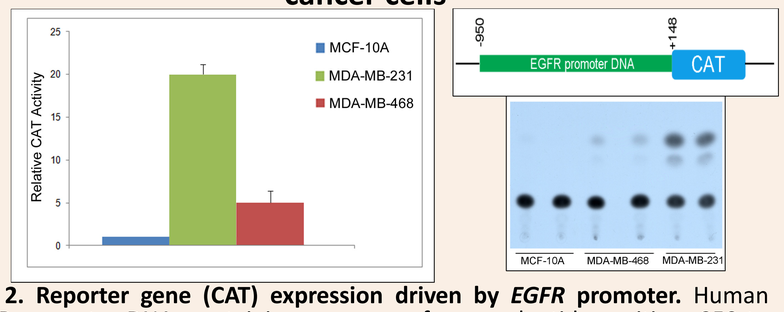

Fig. 2. Reporter gene (CAT) expression driven by *EGFR* promoter. Human *EGFR* promoter DNA, containing sequences from nucleotide position -950 to +148, was cloned into a plasmid vector (pBLCAT3) and transfected into three cell lines, as indicated. The results represent induction of CAT activity relative to the normal breast epithelial cells (MCF-10A). An average of three independent experiments are shown. Expression of CAT (chloramphenicol acetyl transferase) is dependent on the presence of *EGFR* promoter and the ability of the cells to induce the promoter function.

Significant induction of EGFR promoter in MDA-MB-231 cells suggests a possible role of Ras oncogene which is present in this cell line.

Ras expression in breast cancer cells

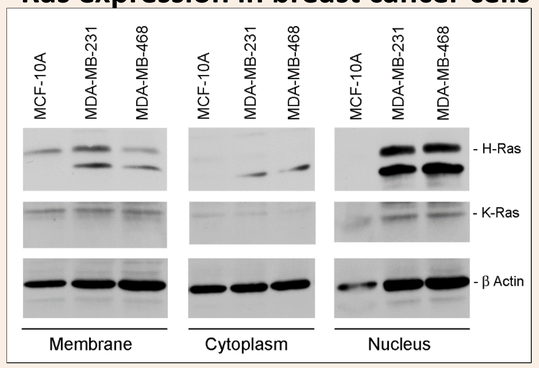

Fig. 3. Western blot analysis of Ras expression. Proteins in the sub-cellular fractions of the indicated cells were fractionated in SDS-PAGE, transferred to a PVDF membrane and blotted with anti-K-Ras, anti-H-Ras and anti-β Actin antibodies (Santa Cruz Biotechnology) in succession. The bands were detected after chemiluminescence reaction.

Presence of both H- and K-Ras in all three cell lines suggests that a mutant Ras (K-RasV12), an activated Ras, which is known to be present in MDA-MB-231 cells might be responsible for the selective induction of EGFR gene (as seen in Fig. 2). Significance of high level of H-Ras in the nucleus of breast cancer cells is yet to be determined.

Ras-mediated Transcription induction of EGFR expression in breast cancer cells

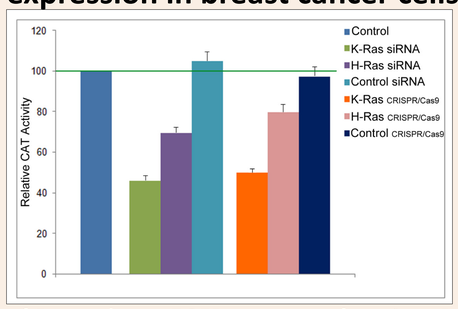

Fig. 4. Depletion of Ras reduces *EGFR* promoter function. Breast cancer cells, MDA-MB-231, were transfected with *EGFR* promoter-containing CAT reporter plasmid. Some of the transfected cells were co-transfected with siRNA to either K-, H-Ras or a control siRNA. Also, in some assays, cells were co-transfected with K-and H-Ras-specific CRISPR/Cas9 (Santa Cruz Biotechnology). The results presented indicate changes in the CAT activity relative to the untreated MDA-MB-231 cells (Control). An average of three independent experiments are shown.

Significant inhibition of K-Ras function suggests a possible role of this oncogene in the induction of EGFR expression.

K-RasV12-SAF1 axis promotes *EGFR* expression

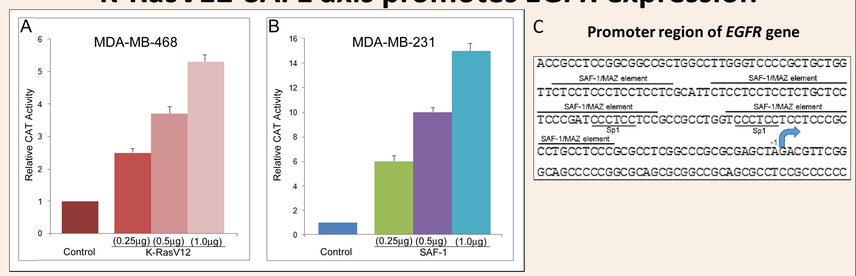

Fig. 5. K-RasV12 and SAF-1 induces EGFR promoter function. A. Breast cancer cells MDA-MB-468 that does not contain a mutant Ras were transfected with *EGFR* promoter-containing CAT reporter plasmid, as described in Fig. 2. Some of the transfected cells were co-transfected with the mutant K-Ras, K-RasV12, plasmid. **B.** MDA-MB-231 cells were co-transfected with SAF-1 expressing plasmid to assess the effect of activated Ras, present in these cells, on SAF-1 in the induction of *EGFR* promoter. In both panels, results present changes in the CAT activity relative to the untreated cells (Control). An average of three independent experiments are shown. **C.** DNA sequence of the *EGFR* promoter shows several SAF-1 binding sites. Transcription start site is indicated by an arrow.

Dose-dependent increase of mutant K-Ras, in panel A, promotes EGFR expression in the low expressing MDA-MB-468 cells (as seen in Fig. 2). Furthermore, over-expression of SAF-1 increases EGFR promoter activity suggesting a possible involvement of SAF-1.

Conclusions

High level of EGFR expression in breast cancer cells is, at least in part, involves a signaling event which is mediated by K-RasV12, a mutant form of K-Ras that is naturally present in the highly metastatic breast cancer cells, MDA-MB-231. High level of *EGFR* promoter activity in MDA-MB-231 (see Fig. 2) explains this phenomenon. Ras signaling activates SAF-1 presumably by the activation of MAP kinase pathway which is known to phosphorylate and activate SAF-1 (6, 7). Activated SAF-1, most likely, binds to the *EGFR* promoter to induce its expression. Multiple SAF-1 binding elements in the *EGFR* promoter (see Fig. 5C) are potential sites for SAF-1 interaction and induction of EGFR. This finding reveals a novel mechanism of *EGFR* expression.

References

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Acknowledgements

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