Characterization of γ-Aminobutyric Acid Type A Receptor–Associated Protein, a Novel Tumor Suppressor, Showing Reduced Expression in Breast Cancer

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Short notes:

- Previous studies have revealed a high frequency of allelic loss on 17p in human cancers.
- In 2001, Seitz and her colleagues showed that there are at least seven commonly deleted regions on chromosome 17p13.1 - p13.3 in sporadic breast cancer.
- Furthermore, transfer of the short arm of chromosome 17 into a *p53* wild-type breast cancer cell line resulted in suppression of tumorigenicity in nude mice.
- These data indicate the presence of at least one more putative tumor suppressor gene in the chromosomal region 17p13, besides the previously described genes *p53* (17p13.1), *Hic-1* (17p13.3), *OVCA1* (17p13.3), and *HCCS1* (17p13.3).

- ɣ-Aminobutyric Acid Type A Receptor–Associated Protein (GABARAP):

- is a cytoplasmic protein of 14 kDa,
- belongs to a protein family which is highly conserved from yeast to mammals,
- located in the chromosomal region 17p13.1.

- Mammalian forms of GABARAP share 100% identity at the amino acid level, suggesting that the function of GABARAP is essential or advantageous in mammals.

- GABARAP shows sequence similarity to light-chain 3 of microtubule associated proteins 1A and 1B at the NH2 terminus, whereas the COOH-terminal part of the protein is thought to interact with the target protein.

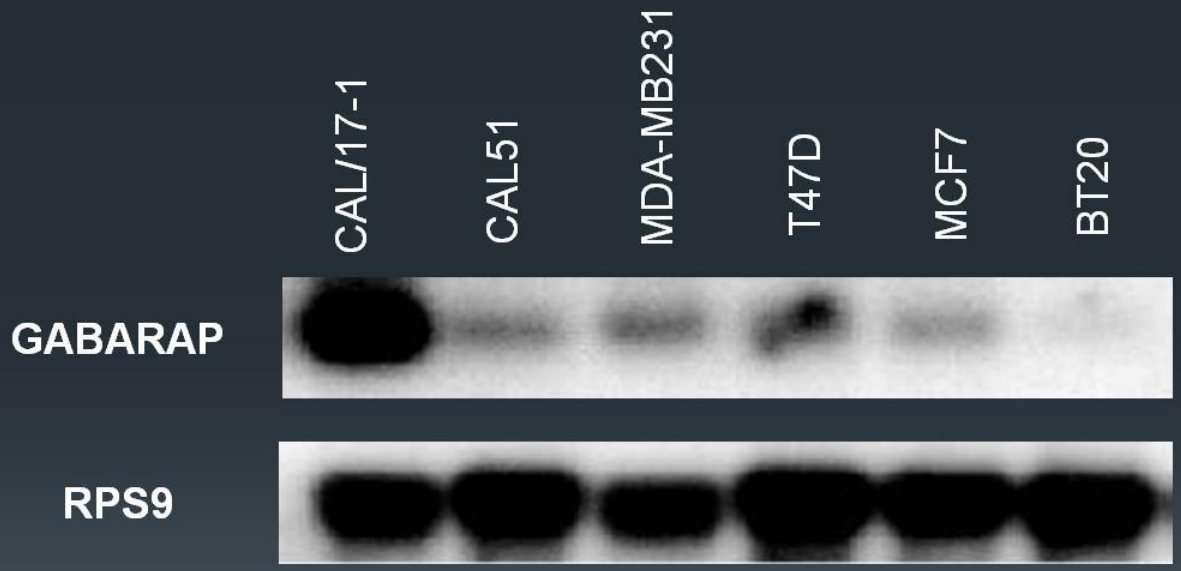
- GABARAP was initially identified as a ligand of the ɣ-subunit of the $GABA_A$ receptor, which is a ligand-gated ion channel that mediates rapid inhibitory synaptic transmission in the central nervous system (CNS) and serves as a target for multiple neuroactive drugs.

- GABARAP acts as a trafficking molecule for different receptors to the plasma membrane like the $GABA_A$ receptor in cortical neurons or the transferrin receptor in HeLa cells.

- Currently, knowledge obtained from the large number of GABARAP binding proteins strongly suggest that it participates in multiple biological processes, such as general vesicular transport and fusion events, autophagy and apoptosis. However, the precise cellular function of GABARAP remains to be elucidated in more detail.

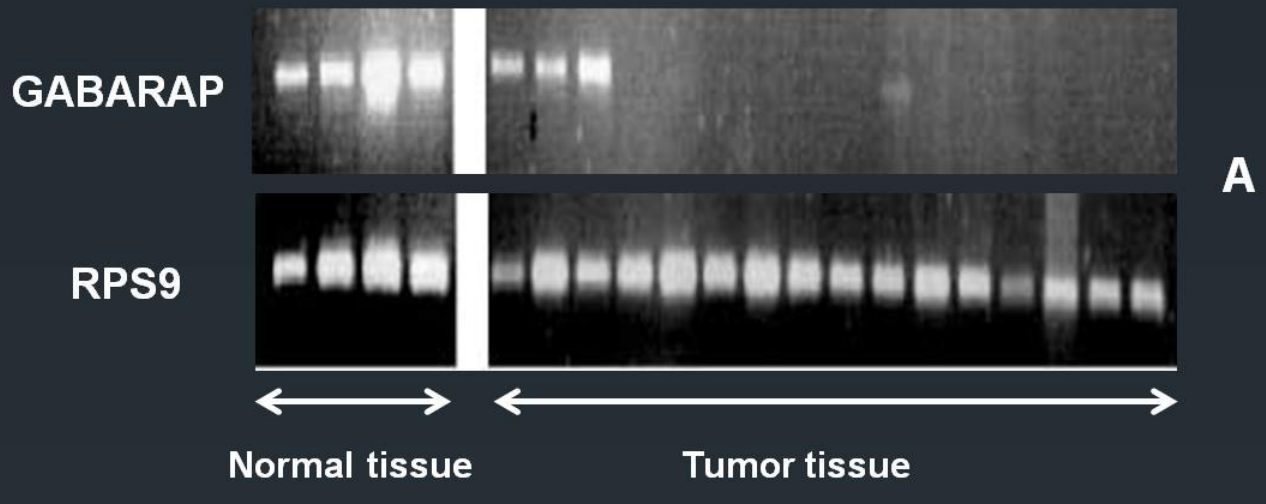
Expression Analysis of GABARAP in Breast Cancer Cell Lines

By performing SSH between the breast cancer cell line CAL51 and the nontumorigenic microcell hybrid CAL/17-1, they constructed one cDNA library representing the genes down-regulated in CAL51. They selected one clone, containing the gene GABARAP for further investigations.



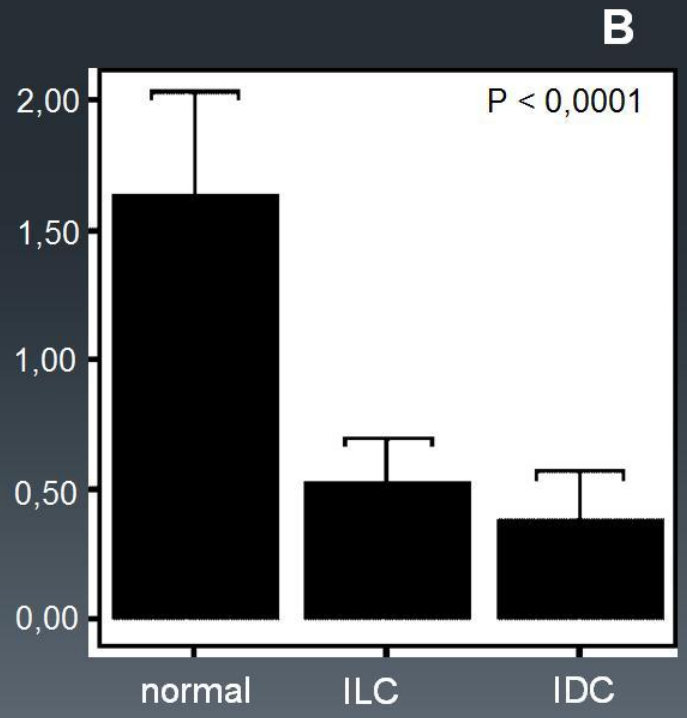
Northern blot analysis of GABARAP from different breast cancer cell lines. Control hybridization was done with ribosomal protein S9 as housekeeping gene.

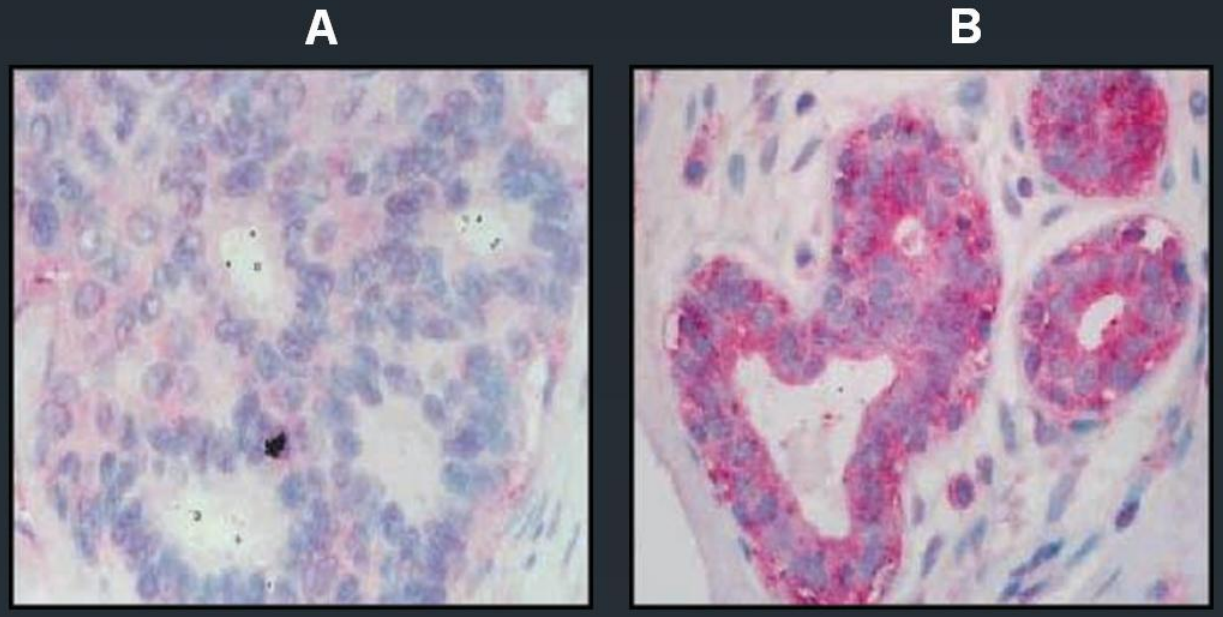
Expression Analysis of GABARAP in Normal and Tumor Tissue



A- *RT-PCR analysis of GABARAP expression in primary breast cancers and normal tissue.*

B- *Diagram of GABARAP mRNA expression in normal breast tissue and invasive lobular (ILC) and invasive ductal (IDC) carcinoma.*



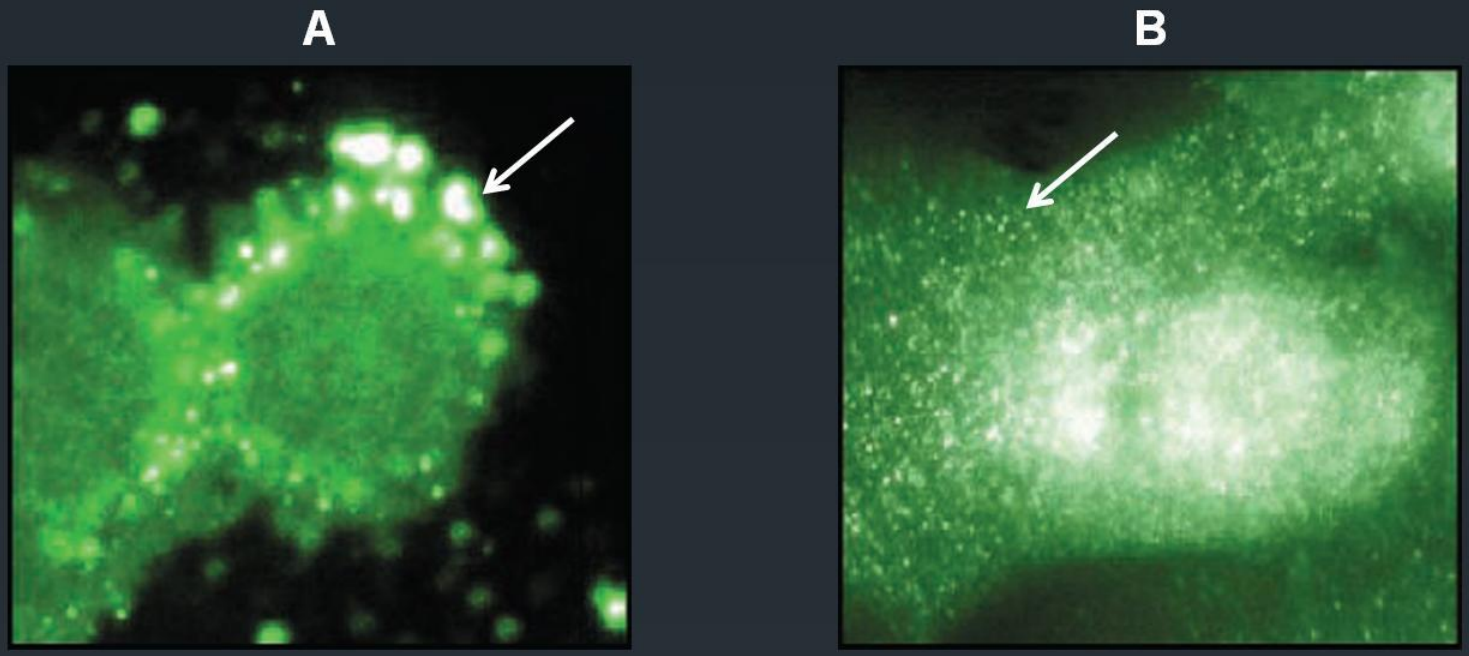


Representative sections with immunohistochemical staining of GABARAP in formalin-fixed, paraffin-embedded, primary breast cancer tissue **(A)** *and normal breast tissue* **(B)**, *with polyclonal anti-GABARAP antibody showing decreased expression in tumor tissue.*

Correlation between GABARAP underexpression and breast tumor specimen detected by immunohistochemistry

	Total	GABARAP-negative, n (%)	GABARAP-positive, n (%)	P
Normal	11	3 (27%)	8 (73%)	
IDC	83 (89%)	53 (57%)	30 (32%)	0.022
ILC	10 (11%)	7 (8%)	3 (3%)	0.050
All tumors	93 (100%)	60 (65%)	33 (35%)	0.017

Subcellular Localization of GABARAP



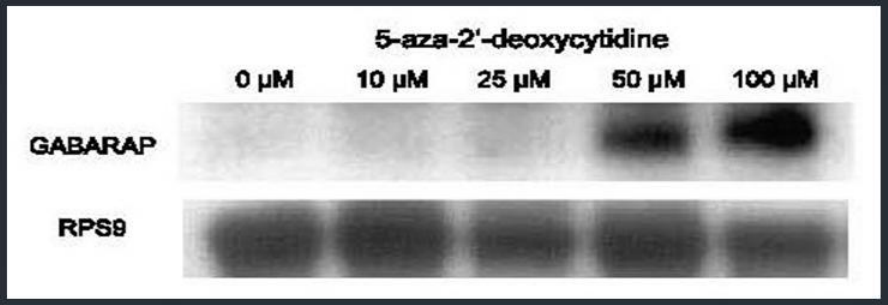
Subcellular localization of GABARAP determined by using immunofluorescent-labeled polyclonal anti-GABARAP antibody. In the microcell hybrid CAL/17-1 with high mRNA expression levels large cytoplasmic vesicles could be detected (A), whereas normal mammary epithelial cells with moderate expression levels showed smaller vesicles (B, arrow).

Mutational Analysis:

Neither mutations nor large interstitial deletions could be observed in the tested tumor cell lines by these investigations (data not shown) indicating that other factors are responsible for transcriptional silencing of GABARAP in tumor cells.

Analysis of Methylation Status

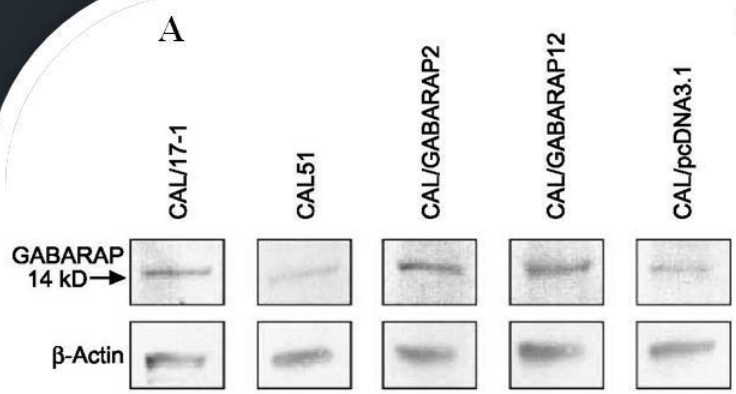
GABARAP expression analysis by Northern blot after treatment with 5-aza-dCyd. Ribosomal protein S9 was used as housekeeping gene.

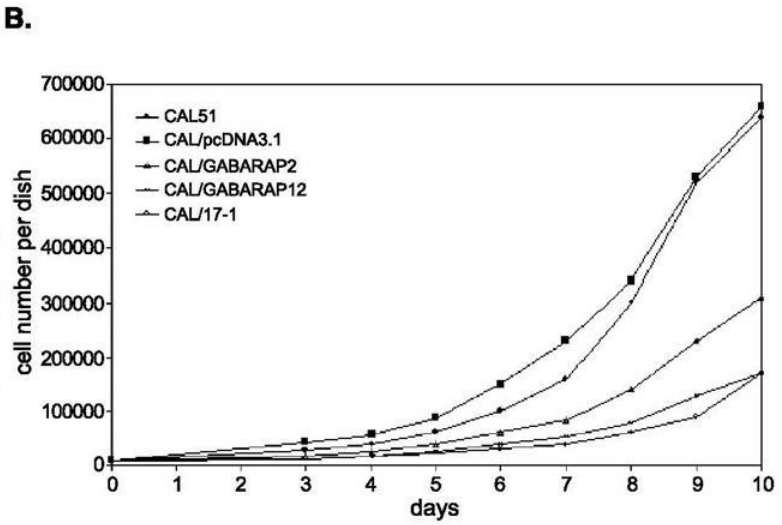


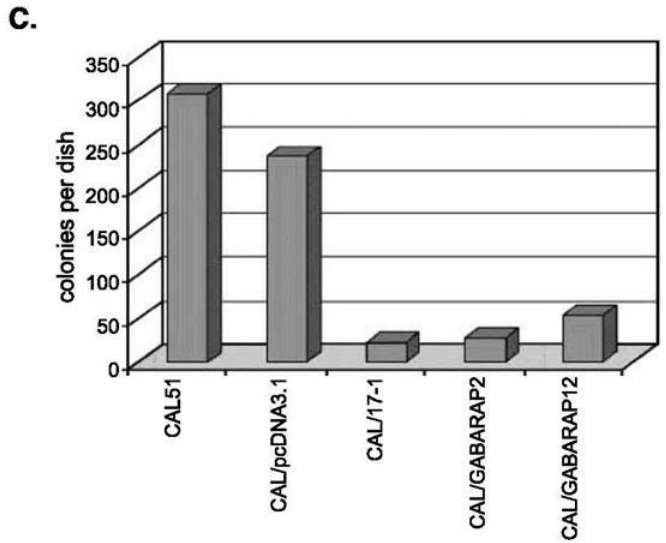
***** No hypermethylation occurred in the promoter region of GABARAP in tumor cells after treatment of DNA with sodium bisulfite.**

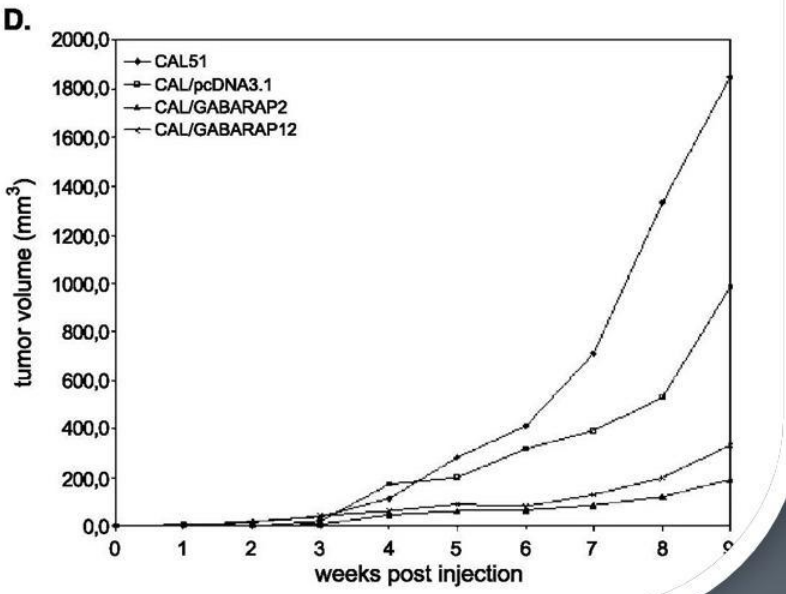
= suggesting a methylation-dependent upstream effect

Characterization of Stable Transfectants









Western blot analysis of GABARAP protein expression in cells stably transfected with full length cDNA of GABARAP showing increased expression of the protein in the GABARAP transfectants compared with the tumor cell line CAL51 and mock-transfected cells CAL/pcDNA3.1. Equal loading of the blot was confirmed by staining with rabbit anti-actin antibody (A). Analysis of the growth rate in vitro (B), colony-forming ability in soft agar (C), and tumorigenicity in immune-deficient nude mice (D) indicated reduced malignant phenotypes of the CAL/GABARAP transfectants.

Conclusion:

These data strengthen the suggestion that GABARAP functions as a putative tumor suppressor gene class II in breast cancer. However, the regulatory pathways of GABARAP gene expression, which lead to down-regulation in tumors, and the precise function of this protein remain to be elucidated.

Thank You for Your Attention