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Systematic characterisation of *GABRP* expression in sporadic breast cancer and normal breast tissue

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The *GABRP* gene has been previously identified by *in silico* analysis of four million ESTs as a candidate gene differentially expressed in breast cancer. *GABRP* is located on chromosome 5q34 and it encodes the π-subunit of the γ-aminobutyric acid (GABA) receptor, a transmembrane protein expressed in the brain and several nonneuronal tissues. Using cDNA dot blot hybridisation (cancer profiling array), quantitative RT-PCR and non-radioisotopic *in situ* hybridisation (ISH), we have analysed *GABRP* expression in breast cancer and normal breast tissues as well as in nontumorigenic and tumorigenic breast cell lines. Analysis of the cancer profiling array revealed a more than 2-fold downregulation of *GABRP* ($p < 0.001$) in 76% of primary breast carcinomas ($n = 50$) compared to corresponding normal tissues. Quantitative RT-PCR in a panel of 23 normal human tissues showed that the *GABRP* expression level was most abundant in the normal breast tissues compared to other human tissues. *GABRP* downregulation in breast cancer was confirmed by quantitative RT-PCR in cryopreserved breast tumour and normal breast tissue specimens ($n = 22$), in archival formalin-fixed, paraffin-embedded tissue specimens ($n = 32$), as well as in breast cancer cell lines ($n = 8$). Furthermore, a significant downregulation of *GABRP* was noted in large (pT3-pT4) ($p = 0.044$) primary breast tumours. Non-radioisotopic ISH showed strong *GABRP* expression in normal epithelial and benign papilloma breast cells, but no signal could be detected in invasive ductal carcinoma. Altogether, these data suggest that *GABRP* is progressively down-regulated with tumour-progression, and that it may be useful as a prognostic marker in breast cancer.

Key words: *GABRP*; gene expression; breast cancer; prognostic marker

The π-subunit of the γ-aminobutyric acid (GABA) receptor, encoded by the gene *GABRP*, is a transmembrane protein expressed in the brain and in several nonneuronal tissues.[1] Type A γ-aminobutyric acid receptors ($GABA_A$ receptors) are heteropentamers, consisting of five subunits that form a chloride ion channel, and mediate the majority of fast inhibitory neurotransmission in the brain.[2] A wide range of $GABA_A$ receptor subunit classes have been discovered in recent years: α(1-6), β(1-3), γ(1-3), δ, ε, θ, π.[1–5] Alternative splicing further increases the repertoire of $GABA_A$ receptors.[1–4] Depending on their subunit composition, $GABA_A$ receptors have different pharmacological and electrophysiological properties. The subunit diversity results in a very large number of potential subunit combinations and, thus, in a large number of possible $GABA_A$ receptor subtypes.[6]

The majority of genes encoding $GABA_A$ receptor subunits are found in four clusters on human chromosomes 4, 5, 15 and X.[2,7,8] *GABRP* is located on chromosome 5q34, but it is not tightly linked with the $GABA_A$ receptor gene cluster.[9] The amino acid sequence of the π-subunit is most closely related to $GABA_A$ receptor β- and δ-subunits and to the $GABA_C$ receptor ρ-subunit, and is less similar to other $GABA_A$ receptor or glycine receptor subunits.[1] The polypeptide of *GABRP* has all of the hallmarks of a ligand-gated anion channel subunit. Of the 78 amino acid residues that are conserved between all known $GABA_A$ receptor subunits only 6 are substituted in the π-subunit sequence.[1]

The π-subunit has been previously amplified from cDNA libraries from two brain regions (hippocampus and temporal cortex) with RT-PCR, but no transcripts were hybridised from whole brain samples with Northern blot analysis.[1] It is also expressed by the teratocarcinoma NT2 neuronal precursor cells[1] and terminally differentiated NT2-N cells after treatment with retinoic acid.[10] In nonneuronal tissues, the π-subunit has been amplified from cDNA libraries from the uterus, prostate, ovaries, placenta, gall bladder, lung and small intestine.[1] The precise function of $GABA_A$ receptors, including the π-subunit, in nonneuronal tissues is presently poorly understood.

We have previously used the *in silico* method electronic Northern (eNorthern) for RNA expression profiling to identify hundreds of candidate genes differentially expressed in breast and ovarian cancers.[11] A set of 40 candidate genes identified with this approach has already been characterised by cDNA dot blot using cancer profiling array, by real-time RT-PCR and by RNA *in situ* hybridisation (ISH).[12,13] *In silico* expression data have also led to the identification of *GABRP* among other candidate genes, prompting its further characterisation.[11] We present herein a systematic characterisation of *GABRP* expression in normal breast tissue and breast cancer, using cDNA dot blot hybridisation (cancer profiling array), quantitative RT-PCR and non-radioisotopic ISH.

Material and methods

Tissues specimens

Cryopreserved tissue specimens from 6 breast tumours and matched normal breast tissue and 10 additional unmatched breast tumours were obtained from patients treated at the gynecology department of the University Hospital Charité-Berlin in Germany. Tissue specimens were snap-frozen in liquid nitrogen immediately after surgery. Haematoxylin and eosin-stained sections were prepared for assessment of the percentage of tumour cells, and only samples with greater than 80% tumour cells were selected for analysis. Frozen tissue samples were homogenised in liquid nitrogen and dissolved in lysis buffer, followed by RNA extraction, using Trizol (Life Technologies, Mannheim, Germany), according to the protocol supplied by the manufacturer.

Archival formalin-fixed, paraffin-embedded tissue from 21 breast cancer and 11 unmatched normal breast tissue specimens were

Abbreviations: DEPC, Diethylpyrocarbonate; EST, expressed sequence tag; GABA, γ-aminobutyric acid; GABRP, π-subunit of the γ-aminobutyric acid receptor; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; ISH, *in situ* hybridisation; RT-PCR, reverse transcription–polymerase chain reaction.

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TABLE I – PRIMERS AND PROBES USED IN REAL-TIME RT-PCR

Gene	Primer sequence	Product size (bp)	TaqMan probe
GABRP	5′-CAGCCAACAGTACCAAAAGTGATT-3′*†	85	5′-TTGAGTGTGCCAGGGTAAAGGCTTCCA-3′
	5′-TGGCGAGATTGTCTAAAATAACTGA-3′*†		
GAPDH	5′-GAAGGTGAAGGTCGGAGTC-3′*	226	5′-CAAGCTTCCCGTTCTCAGCC-3′
	5′-GAAGATGGTGATGGGATTTC-3′*		
GAPDH	5′-TGGTCACCAGGGCTGCTT-3′†	151	
	5′-AGCTTCCCGTTCTCAGCCTT-3′†		

*Primers for RT-PCR in cryopreserved tissue specimens.–†Primers for RT-PCR in formalin-fixed, paraffin-embedded tissue specimens.

obtained from patients treated at the gynecology department of the University Hospital of Aachen, Germany. For each formalin-fixed, paraffin-embedded tissue specimen, six 4-µm thick tissue sections were cut with a microtome (Leica Microsystems, Leica, Germany) and transferred to a water bath filled with DEPC-treated water. Sections were mounted on standard glass slides and dried for 1 hr at 60°C. Sections were deparaffinised and rehydrated as follows: 2 × 15 min in xylole, 2 × 15 min in 100% ethanole and short rinses in 96, 70 and 50% ethanole, followed by emersion in distilled water. Tissue material was transferred to a microcentrifuge tube and RNA was extracted, according to the Trizol protocol supplied by the manufacturer (Life Technologies).

Cell lines

The nontumorigenic cell line MCF10A and 7 tumorigenic cell lines (MCF7, SKBR3, T47D, ZR75-1, BT-20, MDA-MB453 and MDA-MB468) were obtained from the Institute of Pathology, University Hospital Aachen and cultured as described in ATCC cell biology catalogue (LGC Promochem, Teddington, England).

Expression analysis using the cancer profiling array

The matched tumour/normal expression array used consists of 103 cDNAs synthesised from human tumorigenic and corresponding normal tissue, *i.e.*, 50 breast cancer, 50 normal breast tissue and 3 breast tumour lymph node metastasis specimens. Each pair was independently normalised based on the expression of four housekeeping genes (ubiquitin, 23 kDa highly basic protein, β-actin and glutamate dehydrogenase) and immobilised in separate dots. Histological type, patient age and a complete list of tissues can be found on the provider's website (www.clontech.com/clontech/techinfo/manuals/PDF/pt7841–1.pdf). Hybridisation using 25 ng of a gene-specific ^{32}P-labelled cDNA probe digested from Unigene cDNA clone (Accession Number AA101225) was performed according to the manufacturer's recommendations. The tumour/normal intensity ratio was calculated using a Storm-860 phosphoimager (Molecular Dynamics, Eugene, OR) and normalised against the background.

Quantitative RT-PCR

GABRP expression was analysed with real-time RT-PCR in a panel of 23 normal human tissues (see Fig. 2), using commercially available RNA (Clontech, Heidelberg, Germany), and in 54 normal and malignant breast tissue samples.

Real-time RT-PCR was performed using the Gen Amp® 5700 sequence detection system (PE Applied Biosystems, Weiterstadt, Germany), using intron-spanning primers and FAM (5′ end)/TAMRA (3′ end)-labelled specific oligonucleotides. The housekeeping gene *GAPDH* was used as reference. Primers and probes used in this study are presented in Table I. Each PCR reaction was performed in a 25 µl volume that included 12.5 µl 2× TaqMan Universal PCR-Mastermix (PE Applied Biosystems), 1 ng of cDNA template, 300 nM of each primer and 100 nM of the specific probe. Gene expression was quantified by the comparative C_T method, normalising C_T values to the housekeeping gene *GAPDH* and calculating the relative expression values of tumour and normal tissues.[14]

GABRP mRNA expression was further analysed with the LightCycler® system (Roche Diagnostics, Mannheim, Germany) in archival formalin-fixed, paraffin-embedded breast cancer and normal breast tissue specimens, as well as in nontumorigenic and tumorigenic breast cell lines. *GAPDH mRNA* was used as reference to obtain relative expression values. *GABRP* primers were the same as those used for RT-PCR in cryopreserved tissue specimens. New *GAPDH* primers with smaller product size were designed to optimise RT-PCR on formalin-fixed, paraffin-embedded tissue (Table I). Real-time RT-PCR was carried out with Fast Start DNA master hybridisation probes (Roche Molecular Biochemicals, Mannheim, Germany). The conditions were as follows: initial denaturation in 1 cycle of 15 min at 95°C, followed by 40 cycles at 95°C for 20 sec, 60°C for 20 sec and 72°C for 30 sec. Reaction, data acquisition and analysis were all done by using the LightCycler® instrument.

Non-radioisotopic RNA ISH

Non-radioisotopic RNA ISH was performed as previously described.[12] In brief, riboprobes were obtained from plasmids containing cDNA inserts from the same clones used for array hybridisation, linearised with restriction enzymes. Probes were digoxigenin-labelled using the Dig RNA labelling kit (Roche Applied Science, Mannheim, Germany). Paraffin-embedded tissue specimens were deparaffinised, rehydrated, washed two times in PBS, and processed according to the manufacturer's instructions (Roche Applied Science). Hybridised probes were detected using alkaline phosphatase-conjugated anti-DIG antibodies and BM Purple as substrate (Roche Applied Science). After nuclear fast red counter staining (containing 5% aluminium sulphate; VWR International, Dublin, Ireland), sections were examined by a pathologist.

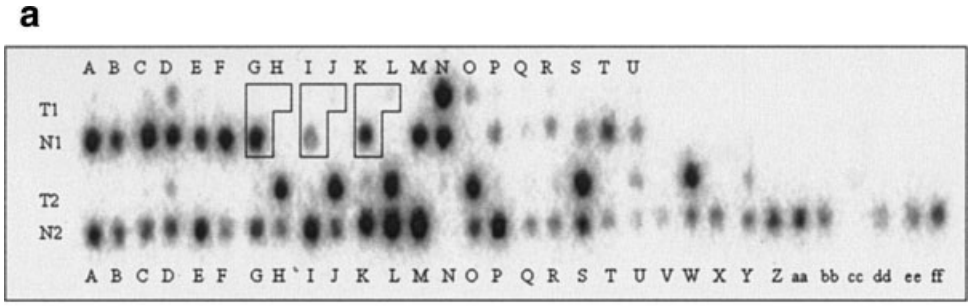
Statistical analysis

To compare the delta CT values of the real-time RT-PCR results between specific groups, the nonparametric Mann-Whitney U test was used. A 2-sided Wilcoxon test for dependent variables was calculated to compare delta CT values of matched normal and tumour samples, *i.e.*, the cancer profiling array data and the 6 matched cryopreserved tissue samples analysed with real-time RT-PCR.

Results

Expression analysis using the cancer profiling array

In silico expression data of *GABRP* were initially validated by dot blot analysis using a cancer profiling array containing 103 cDNAs from 50 breast cancer patients, *i.e.*, from 50 primary breast cancer, 50 normal breast tissue and 3 breast cancer lymph node metastasis specimens. Results of the dot blot analysis are presented in Figure 1*a*. Cancer profiling array analysis showed downregulation of *GABRP* in 38 out of 50 primary breast tumours (76%), as well as in all 3 metastatic lymph nodes, as compared to matched normal breast tissue. *GABRP* was upregulated in 8 out of 50 primary breast tumours (16%) as compared to corresponding normal tissue, while in 4 cases (8%) there was no difference in *GABRP* expression between the tumour and the matched normal breast tissue probe. Statistical analysis with a 2-sided Wilcoxon test for dependent variables showed that *GABRP* downregulation in breast tumours was statistically significant ($p < 0.001$) (Fig. 1*b*). No correlation was found between the *GABRP* expression pattern and available clinico-pathological data (histological type and patient age).



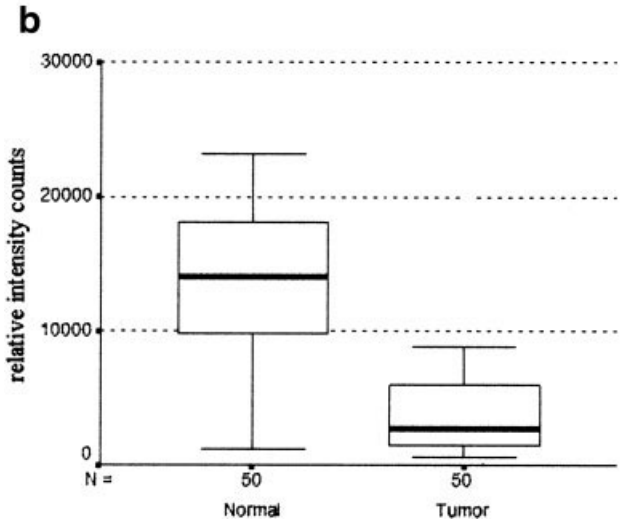


FIGURE 1 – (*a*) Downregulation of *GABRP* in breast cancer. Expression profiles were determined using the Clontech cancer profiling array containing cDNA pairs derived from 50 primary breast cancer, 50 normal breast tissue and 3 breast tumour lymph node metastasis specimens. Rows T1 (A to U) and T2 (A to Z and aa to ff) represent breast cancer and rows N1 (A to G, I, K and M to U) and N2 (A to Z and aa to ff) represent normal breast tissue. The outlined groups represent primary tumour (T1-G, T1-I and T1-K), metastatic lymph nodes (T1-H, T1-J and T1-L) and normal breast tissue (N1-G, N1-I and N1-K) from the same patient. (T, Tumour; N, Normal). (*b*) Box blots showing raw intensity values of the dot blot analysis (Fig. 1*a*): *GABRP* downregulation in breast tumours ($n = 50$) compared to matched normal breast tissue ($n = 50$) ($p < 0.001$, 2-sided Wilcoxon test for dependent variables). Horizontal lines: group medians; boxes: 25–75% quartiles, range, peak and minimum.

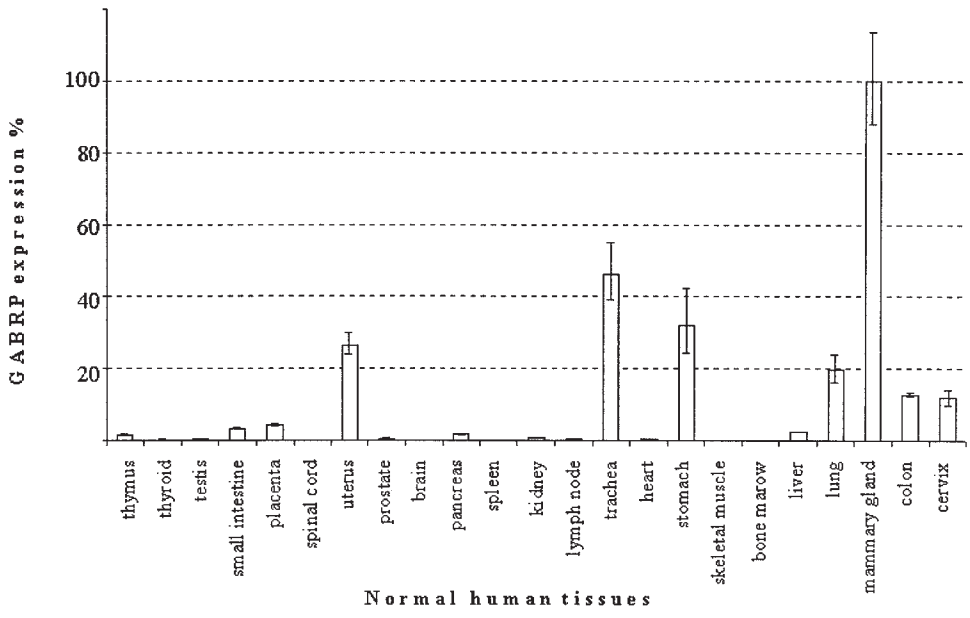


FIGURE 2 – Abundant *GABRP* expression in human normal mammary gland. Diagrammatic presentation of real-time PCR data demonstrating the level of *GABRP* mRNA expression in human mammary gland and 22 further normal human tissues. Commercially available poly A^+ RNA (Clontech) was analysed. cDNAs were synthesised as described in Material and methods. Among all normal human tissues tested, normal breast tissue exhibited the highest level of *GABRP* expression (set equal to 100%). Notably, there was no detectable *GABRP* expression in whole brain extracts, confirming previously published data.[1]

Quantitative RT-PCR

GABRP expression was analysed with real-time RT-PCR in a panel of 23 normal human tissues, using commercially available RNA. Among all normal human tissues tested, normal breast tissue exhibited the highest level of *GABRP* expression, followed in descending order by trachea, stomach, uterus, lung, colon and cervix. *GABRP* expression was minimal in the following tissues: placenta, small intestine, liver, thymus, pancreas, prostate, heart and kidney. Consistent with previously published data,[1] no *GABRP* expression was detected in RNA from whole brain extracts. Likewise, there was no detectable *GABRP* expression in the following tissues: thyroid, testis, spinal cord, spleen, lymph node, skeletal muscle and bone marrow. Real-time RT-PCR data from the panel of normal human tissues are diagrammatically presented in Figure 2.

GABRP expression was further validated with real-time RT-PCR using RNA extracted from 22 cryopreserved tissue samples,

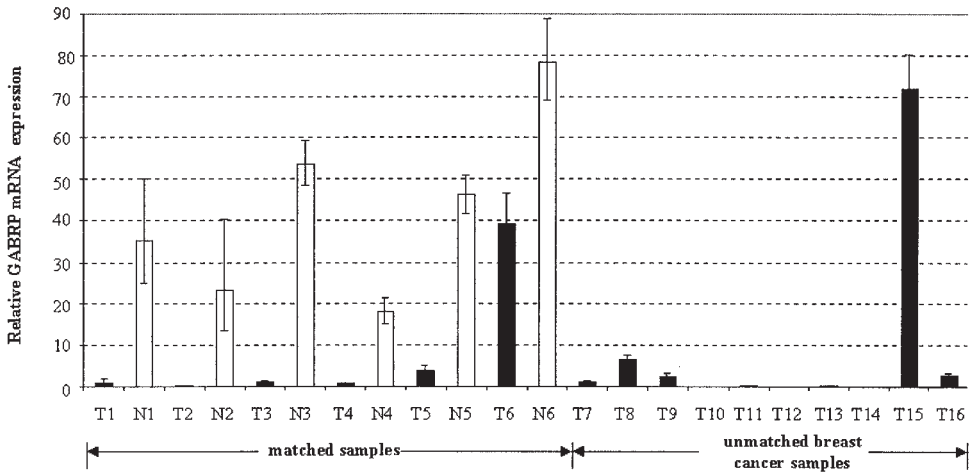


FIGURE 3 – Diagrammatic presentation of real-time RT-PCR data for *GABRP* mRNA from cryopreserved breast cancer and normal breast tissue specimens. *GABRP* was downregulated in 6 matched breast tumours (T1-T6) as compared to corresponding normal breast tissue (N1-N6). *GABRP* expression was minimal in 9 out of ten unmatched breast tumours (T7-T14 and T16), while 1 breast tumour (T15) expressed *GABRP* at a level comparable to normal breast tissues. (T, Tumour; N, Normal).

namely 6 primary breast tumours and 6 matched normal breast tissue and 10 additional unmatched primary breast cancer specimens. Downregulation of *GABRP* was confirmed in all 6 matched breast tumours as compared to corresponding normal breast tissue. Statistical analysis with a 2-sided Wilcoxon test for dependent variables showed that *GABRP* downregulation in the 6 matched samples was statistically significant ($p = 0.031$). Furthermore, *GABRP* expression was minimal in 9 out of 10 unmatched breast tumours, with only 1 breast tumour expressing *GABRP* at a level comparable to normal breast tissues. Real-time RT-PCR data from cryopreserved tissue samples are diagrammatically presented in Figure 3.

Since accurate clinico-pathological data concerning the cryopreserved tissue samples analysed with real-time RT-PCR were incomplete, we have further analysed *GABRP* expression in a set of archival formalin-fixed, paraffin-embedded tissue specimens. This set of archival tissue specimens consisting of 21 primary breast cancer and 11 normal breast tissue samples were analysed with LightCycler® RT-PCR. These data are diagrammatically presented in Figure 4*a*. Consistently with the cancer profiling array and real-time RT-PCR results presented earlier, mean *GABRP* expression in archival tissue samples was more than 2-fold downregulated in breast tumours as compared to normal breast tissues; this difference was statistically significant ($p = 0.002$, Mann-Whitney U test) (Fig. 4*b*). Very interestingly, the *GABRP* expression level was significantly lower in pT3-pT4 as compared to pT1-pT2 primary breast tumours ($p = 0.044$, Mann-Whitney U test) (Fig. 4*c*), and a nonsignificant trend was noted showing a more marked downregulation of *GABRP* expression in node-positive as compared to node-negative primary breast tumours (Fig. 4*d*). No significant association was found between the *GABRP* expression level and patient age, histological type, histological grading, estrogen and progesterone receptor status and expression of HER2.

To investigate the possibility that *GABRP* might be involved in mammary tumour progression, we have compared GABRP mRNA levels in nontumorigenic and tumorigenic breast cancer cell lines with LightCycler® RT-PCR. We found that *GABRP* is expressed only in the nontumorigenic cell line MCF10A. *GABRP* expression was very low or undetectable in all tumorigenic breast cancer cell lines analysed (Fig. 5).

Cellular localisation of GABRP

Cellular localisation of *GABRP* in breast cancer, benign and normal breast tissue was analysed with non-radioactive ISH ($n = 33$). Consistently with the cancer profiling array and quantitative RT-PCR results presented above, *GABRP* expression was downregulated in breast cancer as compared to normal and benign breast tissue. Representative sections showing *GABRP* expression as detected by ISH are presented in Figure 6. *GABRP* was specifically expressed in normal breast epithelial cells (*a*, *c*), as well as in epithelial cells of benign papilloma (*e*), while no ISH signal was found in most invasive ductal carcinomas analysed (*g*).

Discussion

Non-radioactive ISH experiments presented in our study provide for the first time clear evidence that *GABRP* is physiologically expressed in normal and benign human epithelial cells of the breast. Moreover, *GABRP* expression in the normal breast appears to be markedly higher compared to other normal human tissues, including the CNS. The exact role of *GABRP* expression in the breast is at present unclear. To date, very little is known about the role of *GABRP* and $GABA_A$ receptors in nonneuronal tissues. $GABA_A$ receptor subunit subtypes are differentially expressed throughout development, both in different CNS regions and in nonneuronal tissues.[10,15–17] Their assembly[9,18–20] and stoichiometry[21,22] seem to be highly regulated so that the potential combinations of subunits do not always form pharmacologically and electrophysiologically functional chloride ion channels. It is still unclear whether coassembly of the π-subunit into functional $GABA_A$ receptors occurs in neurons *in vivo*.[9,10] Thus, it has been suggested that *GABRP* expression might play a role in neuronal development and/or regulation of neuronal excitability in the CNS, and a more critical role in the function of peripheral $GABA_A$ receptors in nonneuronal tissues.[23]

However, it is not clear whether *GABRP* protein expressed in nonneuronal tissues always coassembles with other subtypes to form peripheral $GABA_A$ receptors or if *GABRP* might have a $GABA_A$-receptor-independent function. On the basis on our findings, consistently showing *GABRP* downregulation in breast can-

FIGURE 4 – Diagrammatic presentation of quantitative RT-PCR data for *GABRP* mRNA from formalin-fixed, paraffin-embedded breast cancer and normal breast tissue specimens (*a*) and statistical analysis in subgroups (*b–d*). (*a*) Mean *GABRP* expression was more than 2-fold downregulated in breast tumours (samples 1–21) as compared to normal breast tissues (samples A–K). Samples 1–14 are from node-positive breast tumours and samples 15–21 are from node-negative breast tumours. (*b*) Box plots showing *GABRP* expression data in breast tumours ($n = 21$) and normal breast tissue ($n = 11$), indicating a highly significant downregulation of *GABRP* in the tumour tissue ($p = 0.002$, Mann-Whitney U test). (*c*) The *GABRP* expression level was significantly lower in pT3-pT4 as compared to pT1-pT2 primary breast tumours ($p = 0.044$, Mann-Whitney U test). (*d*) *GABRP* expression levels in node-positive as compared to node-negative primary breast tumours ($p = 0.431$, Mann-Whitney U test).

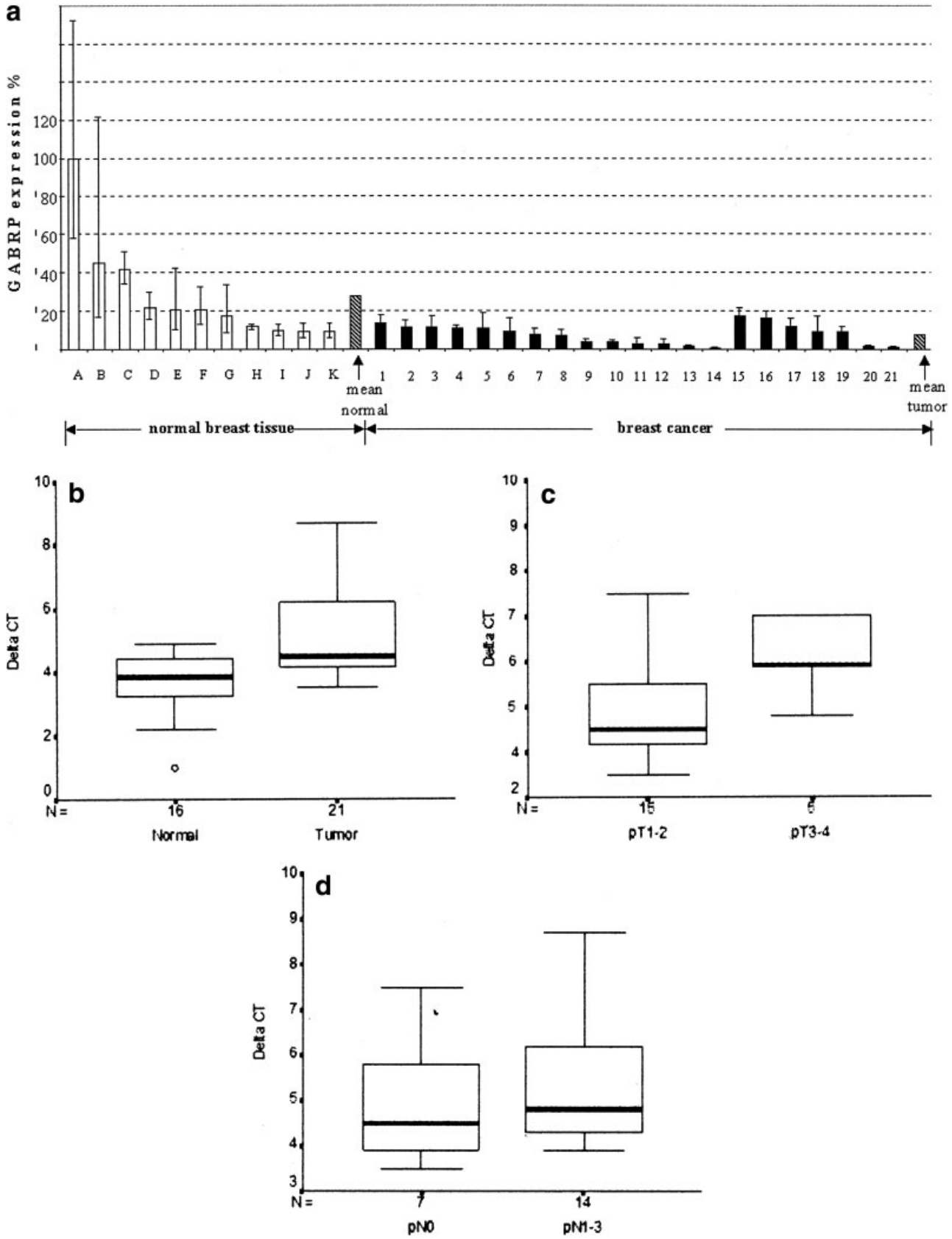


FIGURE 4.

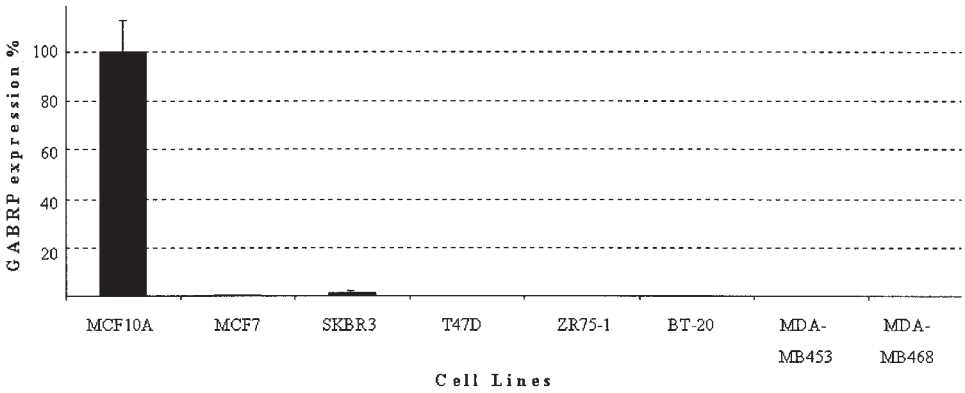


FIGURE 5 – Loss of *GABRP* expression in tumorigenic breast cancer cell lines. Diagrammatic presentation of quantitative RT-PCR data. *GABRP* mRNA was abundantly expressed in the nontumorigenic cell line MCF10A. In tumorigenic breast cancer cell lines, *GABRP* expression was either very low (MCF7, SKBR3) or undetectable (T47D, ZR75-1, BT-20, MDA-MB453 and MDA-MB468).

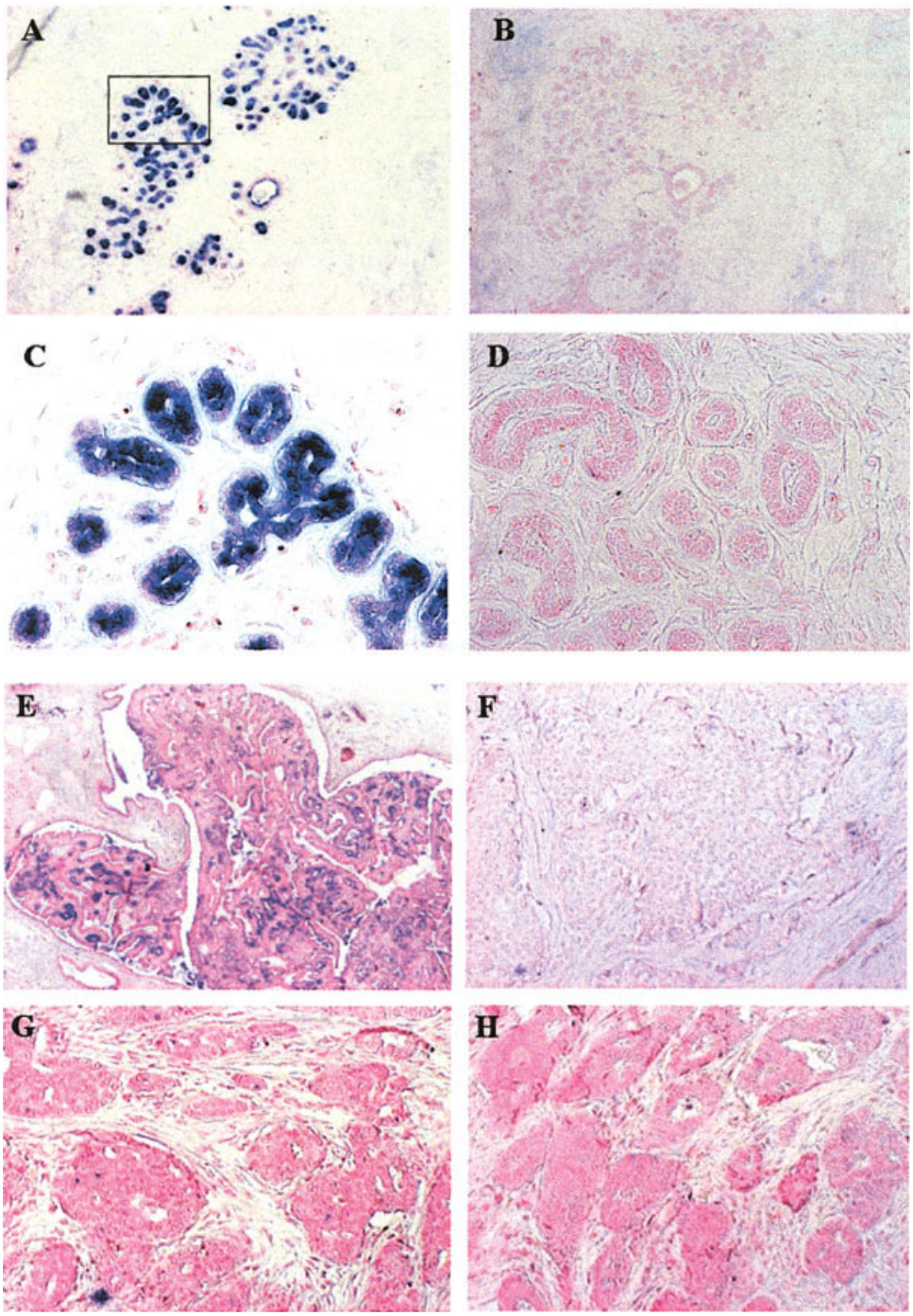


FIGURE 6 – Representative sections of *GABRP* mRNA expression as detected by non-radioactive ISH. (*a*), (*c*), (*e*) and (*g*) Antisense GABRP probe; (*b*), (*d*), (*f*), (*h*) Sense (control) GABRP probe. *GABRP* mRNA was clearly detectable in normal breast epithelial cells (*a* and scale up in *c*) and epithelial cells of breast papilloma (*e*), while no signal was detected in invasive ductal carcinoma sections (*g*).

cer as compared to normal and benign breast tissue with three independent methods, as well as downregulation in tumorigenic as compared to nontumorigenic cell lines, it can be postulated that *GABRP* might exert a tumour-suppressor, $GABA_A$-receptor-independent function in breast tissue. The more marked downregulation of *GABRP* with tumour progression found in our study, *i.e.*, in pT3-pT4 and possibly in node-positive tumours, is consistent with this hypothesis. These associations also suggest that *GABRP* could be possibly used as a tissue marker with prognostic significance in breast cancer. On the other hand, *GABRP* downregulation might be merely an epiphenomenon, a consequence of tumour progression, rather than a cause.

It has been previously proposed that *GABRP* could be used in multi-marker assays as a diagnostic marker for breast cancer metastases in lymph nodes, particularly in sentinel lymph nodes.[24] A diagnostic marker for cancer should be ideally upregulated in the majority of tumour samples, whereas *GABRP*, as shown in the present study, is downregulated in most breast tumours as compared to normal breast tissue, thus limiting its applicability as a single diagnostic marker. Despite this apparent limitation, *GABRP* could still be used, possibly in combination with other markers, in the context of multi-marker diagnostic assays.

$GABA_A$ receptor subtype expression has been previously detected in endocrine responsive tissues other than the breast. $GABA_A$ receptor have been proposed to regulate uterine motility by inhibiting contractions and by mediating the relaxing effects of 5α,3α,-pregnenolone.[1] Furthermore, it has been shown that *GABRP*-mRNA is abundant in the rat uterus throughout pregnancy and falls markedly at term.[17] Other $GABA_A$ receptor subunits also show various fluctuations during pregnancy, suggesting that the resulting receptor with distinct properties may play a role at the onset of labour.[17] In addition, it has been previously postulated that $GABA_A$ receptors in nonneuronal tissues might play a role in the regulation of hormone secretion.[1] Likewise, it can be postulated that *GABRP* expression in the breast might play a role in the mediation of endocrine stimuli.

In conclusion, systematic characterisation of *GABRP* expression with three independent methods consistently showed downregulation of *GABRP* in breast cancer as compared to normal breast tissue, with more marked downregulation in advanced tumour stages. These findings suggest that *GABRP* could be possibly used as a tissue marker with prognostic significance in breast cancer. It is at present unclear if *GABRP* plays a functional role in breast cancer, and this should be evaluated in future studies.

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References

1. Hedblom E, Kirkness EF. A novel class of GABAA receptor subunit in tissues of the reproductive system. J Biol Chem 1997;272:15346–50.
2. Rabow LE, Russek SJ, Farb DH. From ion currents to genomic analysis: recent advances in GABAA receptor research. Synapse 1995;21:189–274.
3. Macdonald RL, Olsen RW. GABAA receptor channels. Annu Rev Neurosci 1994;17:569–602.
4. Davies PA, Hanna MC, Hales TG, Kirkness EF. Insensitivity to anaesthetic agents conferred by a class of GABA(A) receptor subunit. Nature 1997;385:820–3.
5. Bonnert TP, McKernan RM, Farrar S, le Bourdelles B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Brown N, Wafford KA, Whiting PJ. θ, A novel γ-aminobutyric acid type A receptor subunit. Proc Natl Acad Sci USA 1999;96:9891–6.
6. Sieghart W. Structure and pharmacology of γ-aminobutyric acid A receptor subtypes. Pharmacol Rev 1995;47:181–234.
7. McLean PJ, Farb DH, Russek SJ. Mapping of the α_4 subunit gene (GABRA4) to human chromosome 4 defines an α_2–α_4–β_1–γ_1 gene cluster: further evidence that modern GABAA receptor gene clusters are derived from an ancestral cluster. Genomics 1995;26:580–6.
8. Wilke K, Gaul R, Klauck SM, Poustka A. A gene in human chromosome band Xq28 (GABRE) defines a putative new subunit class of the GABAA neurotransmitter receptor. Genomics 1997;45:1–10.
9. Neelands TR, Zhang J, Macdonald RL. GABA(A) receptors expressed in undifferentiated human teratocarcinoma NT2 cells differ from those expressed by differentiated NT2-N cells. J Neurosci 1999;19:7057–65.
10. Neelands TR, Greenfield LJ, Jr, Zhang J, Turner RS, Macdonald RL. GABAA receptor pharmacology and subtype mRNA expression in human neuronal NT2-N cells. J Neurosci 1998;18:4993–5007.
11. Schmitt AO, Specht T, Beckmann G, Dahl E, Pilarsky CP, Hinzmann B, Rosenthal A. Exhaustive mining of EST libraries for genes differentially expressed in normal and tumor tissues. Nucleic Acids Res 1999;27:4251–60.
12. Dahl E, Sadr-Nabavi A, Klopocki E, Betz B, Grube S, Kreutzfeld R, Himmelfarb M, An HX, Gelling S, Klaman I, Hinzmann B, Kristiansen G, et al. Systematic identification and molecular characterisation of genes differentially expressed in breast and ovarian cancer. J Pathol 2005;205:21–8.
13. Klopocki E, Kristiansen G, Wild PJ, Klaman I, Castanos-Velez E, Singer G, Stohr R, Simon R, Sauter G, Leibiger H, Essers L, Weber B, et al. Loss of SFRP1 is associated with breast cancer progression and poor prognosis in early stage tumors. Int J Oncol 2004;25:641–9.
14. Fink L, Seeger W, Ermert L, Hanze J, Stahl U, Grimminger F, Kummer W, Bohle RM. Real-time quantitative RT-PCR after laser-assisted cell picking. Nat Med 1998;4:1329–33.
15. Wisden W, Laurie DJ, Monyer H, Seeburg PH. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 1992;12:1040–62.
16. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development J Neurosci 1992;12:4151–72.
17. Fujii E, Mellon SH. Regulation of uterine γ-aminobutyric acid(A) receptor subunit expression throughout pregnancy. Endocrinology 2001;142:1770–7.
18. Angelotti TP, Macdonald RL. Assembly of GABAA receptor subunits: α_1 β_1 and α_1 β_1 γ_{2S} subunits produce unique ion channels with dissimilar single-channel properties. J Neurosci 1993;13:1429–40.
19. Burgard EC, Tietz EI, Neelands TR, Macdonald RL. Properties of recombinant γ-aminobutyric acid A receptor isoforms containing the α_5 subunit subtype. Mol Pharmacol 1996;50:119–27.
20. Saxena NC, Macdonald RL. Assembly of GABAA receptor subunits: role of the δ subunit. J Neurosci 1994;14:7077–86.
21. Chang Y, Wang R, Barot S, Weiss DS. Stoichiometry of a recombinant GABAA receptor. J Neurosci 1996;16:5415–24.
22. Tretter V, Ehya N, Fuchs K, Sieghart W. Stoichiometry and assembly of a recombinant GABAA receptor subtype. J Neurosci 1997;17:2728–37.
23. Neelands TR, Macdonald RL. Incorporation of the π subunit into functional γ-aminobutyric acid(A) receptors. Mol Pharmacol 1999;56:598–610.
24. Zehentner BK, Dillon DC, Jiang Y, Xu J, Bennington A, Molesh DA, Zhang X, Reed SG, Persing D, Houghton RL. Application of a multigene reverse transcription-PCR assay for detection of mammaglobin and complementary transcribed genes in breast cancer lymph nodes. Clin Chem 2002;48:1225–31.