Annexin A8 is up-regulated during mouse mammary gland involution and predicts poor survival in breast cancer

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Abstract Purpose: Microarray studies have linked Annexin A8 RNA expression to a “basal cell–like” subset of breast cancers, including BRCA1-related cancers, that are characterized by cytokeratin 5 (CK5) and CK17 expression and show poor prognosis. We assessed Annexin A8’s contribution to the overall prognosis and its expression in normal, benign, and cancerous tissue and addressed Annexin A8’s physiologic role in the mammary gland.

Experimental Design: Using microarrays and reverse transcription-PCR, the Annexin A8 expression was studied during mouse mammary gland development and in isolated mammary structures. Reverse transcription-PCR on cultured human luminal and basal cells, along with immunocytochemistry on normal and benign breast tissues, was used for cellular localization. Annexin A8’s prognostic relevance and its coexpression with CK5 were assessed on tissue arrays of 1,631 cases of invasive breast cancer. Coexpression was further evaluated on a small cohort of 14 BRCA1-related breast cancers.

Results: Annexin A8 was up-regulated during mouse mammary gland involution and in pubertal ductal epithelium. Annexin A8 showed preferred expression in cultured basal cells but predominant luminal expression in normal human breast tissue in vivo. Hyperplasias and in situ carcinomas showed a strong staining of basal cells. Annexin A8 expression was significantly associated with grade (P < 0.0001), CK5 (P < 0.0001), and estrogen receptor status (P < 0.0001); 85.7% BRCA1-related breast tumors coexpressed Annexin A8 and CK5.

Conclusion: Annexin A8 is involved in mouse mammary gland involution. In humans, it is a luminal expressed protein with basal expression in cell culture and in hyperplasia/ductal carcinoma in situ. Expression in invasive breast carcinomas has a significant effect on survival (P = 0.03) but is not independent of grade or CK5.

Annexins belong to a large family of calcium- and phospholipid-binding proteins (1) comprising of four or more copies of annexin repeats responsible for the calcium-regulated membrane-binding activity (2). The individual physiologic functions are very diverse, as recently reviewed (2, 3). Annexin A8 has first been described as an anticoagulation factor (VAC-β; ref. 4) but has also been associated with murine palatogenesis (5) and found associated with lung lamellar bodies (6). It shows the typical inhibitory activity towards phospholipase A2 (4), and its crystal structure has recently been revealed (7). Several annexins, including Annexin A8, have been associated with cancers (2, 8–14). Recent microarray studies have shown a link between Annexin A8 RNA expression and a “basal cell–like” subtype of breast cancers with poor prognosis (15–17). This subtype had been described on the basis that these tumors expressed a group of genes that was expressed in basal cell–derived human cell lines but not in luminal cell–derived or endothelial cell lines. In these studies, Annexin A8 clustered closely together with cytokeratin 5 (CK5) and CK17, two markers of poor prognosis in lymph node negative breast tumors (18). However, the contribution of Annexin A8 and other genes in this cluster to the overall prognosis is as yet unknown.

We therefore studied Annexin A8 in mice as well as in normal, benign, and malignant breast tissue to determine its biological and molecular properties in the mammary gland and assessed the prognostic relevance of Annexin A8 in a large group of patients with invasive breast cancer. Using our microarray database of 18 stages of mammary gland development (19) and reverse transcription-PCR (RT-PCR) analysis, we examined...
Annexin A8 expression during mouse mammary gland development and in human mammary epithelial cells. A, RNA expression profile for Annexin A8 derived from microarray analysis. Bars, SEM for each triplicate time point. The x-axis describes the individual developmental stages (virgin, pregnancy, lactation, and involution) as described in Materials and Methods. The y-axis describes the averaged scaled signals of the triplicate samples for each time point. B, semiquantitative RT-PCR on additional independent RNA samples. C, Annexin A8 microarray data, using total RNA from isolated TEBs (TEB), ducts (DUCT), or empty fat pad (FAT) from 6-week-old virgin mice. D, semiquantitative RT-PCR for Annexin A8 using total RNA from isolated ducts and TEBs. E, Annexin A8 microarray data, using total RNA from isolated mammary gland strips that were either rich in ducts and TEBs (POST), or in ducts (PRE), from 6-week-old virgin mice. F, semiquantitative RT-PCR for Annexin A8, β-sm actin (β-sm actin), and GAPDH on total RNA from selected and cultured human luminal and basal epithelial cells.

Reverse transcription-PCR. Ten micrograms of total mouse RNA from a fourth independently collected mammary gland were treated with DNase (DNA-free; Ambion, Huntingdon, Cambridgeshire, United Kingdom) according to the manufacturer’s protocol and resuspended in 100 µl of H2O. Ten microliters of this solution were used to produce cDNA, using SuperscriptII (Invitrogen, Paisley, United Kingdom) according to the manufacturer’s protocol. One-microliter cDNA was used per PCR reaction using Hot-Star Taq-polymerase (Qiagen, Hilden, Germany). For RT-PCR of mouse mRNA, an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) was used for the chain reaction, using 25 to 30 PCR cycles (30 seconds at 90°C, 30 seconds at 55-59°C, and 30 seconds at 72°C).

The following oligonucleotides were used: mAnnexin A8, 5'-ACACAGATGCCCCTTGGCTCTG-3' and 5'-CTTCGCAACGTCTACGATG-3'; β-actin, 5'-T(J/T)GTGATGGACTCCG(A/T)GAC-3' and 5'-C(G/A)CCACAGCAGTGTTG-3'.

Human luminal and basal cell isolation, RNA isolation, and RT-PCR have been described previously (21, 22). The following oligonucleotides have been used: hAnnexin A8, 5'-GATACGAAGCCAAGGAGCA-3' and 5'-TCCACAAAGCTGCTCAGAC-3'; hβ-actin, 5'-T(J/T)GTGATGGACTCCG(A/T)GAC-3' and 5'-C(G/A)CCACAGCAGTGTTG-3'; hGAPDH, 5'-ACCCACTCCTCACCATTG-3' and 5'-CTCTTGCTGCTCTGCT-3'.

Immunocytochemistry. For Annexin A8, a goat antibody was used (C-20; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:100. Two antibodies were used that recognized CK5. For our studies on benign breast disease, the pilot study, and the BRCA1-related tumors, we used an antibody from Novacastra (XM26; Newcastle upon Tyne, United Kingdom) at 1:300, which is specific for CK5 and shows a similar staining pattern to that observed with an antibody against its heterodimer-binding partner CK14.7 For the tissue array study we used 7 Unpublished data.
the anti-CK5/6 antibody (Boehringer Mannheim, Mannheim, Germany) at 1:50, which has previously been used in breast cancer studies so that the data would be comparable with other data in the literature. For Annexin A8, paraffin-embedded tissue sections were stained using the three-step streptavidin-biotin system incorporating antigen retrieval with EDTA. Endogenous biotin was blocked using the avidin/biotin blocking system (Vector Labs, Burlingame, CA); Fc-γ receptors were preblocked using 10% normal rabbit serum. Binding of horseradish peroxidase-streptavidin was always developed by 3,3'-diaminobenzidine tetrahydrochloride and the tissue was counterstained with hematoxylin. The Envision system (Dako, Carpinteria, CA) was used for CK5 and CK5/6. Antibiot retrieval was done using high pressure and EDTA, pH 8 (CK5, Annexin A8) or citrate buffer, pH 2 (CK5/6) for 5 minutes. Internal peroxidases were blocked with 3% H2O2.

**Human breast tissues.** As an initial study, normal, benign, and malignant lesions were examined. All cases were anonymized and the studies had the Research Ethics Committee approval. The following cases were examined: 21 normal reduction mammoplasties, 25 radial scars with hyperplasia, seven cases of intra luminal hyperplasia of usual type, seven cases of atypical ductal hyperplasia, three cases of grade 1 ductal carcinoma *in situ* (DCIS), nine cases of grade 2 DCIS, eight cases of grade 3 DCIS, 13 cases of lobular carcinoma *in situ*, two medullary carcinomas, one papillary carcinoma, 10 infiltrating lobular carcinomas, 13 mucinous carcinomas, 11 grade 1 ductal carcinomas, two grade 2 ductal carcinomas, and 14 BRCA1-related breast carcinomas. These were an anonymized data set with no clinical follow-up information available.

**Tissue arrays.** The tissue arrays were made at the University Hospital Basel by G.S., representing 2,200 cases of invasive breast carcinoma; 568 cases were not analyzed for technical reasons. One additional case had to be omitted for reasons of missing follow-up information, leaving an evaluable group of 1,631 patients. Additional patient and tumor characteristics for this group of patients included Bloom, Richardson, Elston grade; grade components; histology; age; nodal status; and tumor size group. Histology was provided by a specialist breast pathologist (Joachim Torhorst). Estrogen receptor (ER) and epidermal growth factor receptor status were already known in this cohort.

Immunocytochemistry was done as described above. One pathologist at PathoSuisse AG (Bottighofen, Switzerland) scored all tissue arrays specimens to ensure consistency of interpretation. After a provisional screen of the tissues, a decision was made to use a combination of an intensity score (0, 1, 2, and 3) with percentage of cells positive for the Annexin A8 study. The scoring system used had the following cutoffs: negative, all cells negative; strong, ≥2 in >50% or ≥3 in >20%; weak, between negative and strong. For the CK5 study and in accordance with other studies, any tumors with CK5 positivity within the tumor cells were scored as positive. In both cases, occasional normal myoepithelial cells entrapped in the tumors, which was often seen in association of residual DCIS, were disregarded.

**Statistical analysis.** The 1,631-patient cohort was analyzed for associations with other patient and/or tumor characteristics and effect of Annexin A8 on outcome. The Pearson χ2 test was used to assess associations between the three categories (negative, weak, and strong) of Annexin A8 and other characteristics. Survival was defined as the time from surgery to death from any cause. The median follow-up was 6.6 years. The survival curve was estimated and compared using Kaplan-Meier estimates and log-rank test. The multivariate analyses were done using Cox proportional hazards regression models (23). All Ps were two sided. *Ps < 0.05 were considered statistically significant.*
Results

Annexin A8 mRNA levels during mouse mammary gland development. In an attempt to identify genes involved in different stages of mammary gland development, we recently did microarray studies for over 8,600 genes on 18 stages of development and published a database for mammary gland gene expression. To establish the physiologic role of Annexin A8 in the mammary gland, we studied the RNA expression profile for Annexin A8 in our database. We found that Annexin A8 was one of 96 genes preferentially up-regulated after forced weaning (Fig. 1A). Semiquantitative RT-PCR, using an independent batch of RNA from nine stages of mammary gland development, confirmed the increase in the level of Annexin A8 mRNA 24 hours after pup removal and expression over the first 3 days of mammary gland involution (Fig. 1B). Unfortunately, there were no suitable reagents available to localize Annexin A8 protein in the mouse mammary epithelium. To get an indication as to whether Annexin A8 was expressed in the epithelium or the stroma, we made use of another microarray study, in which we compared the gene expression profiles from isolated TEBs, ducts, and empty fat pad from 6-week-old virgin mice. We detected Annexin A8 mRNA only in the ductal fraction (Fig. 1C) but not in TEBs or in the empty fat pad. This was confirmed by semiquantitative RT-PCR (Fig. 1D). It indicated that Annexin A8 expression was associated with the differentiated epithelium rather than growing epithelium or the stroma. These data were also confirmed in whole tissues, using microarray results obtained from mammary gland strips taken either pre-lymph node (enriched in duct and stroma) or post-lymph node (enriched in TEBs, ducts, and stroma) from 6-week-old virgin mice. Here, the pre-lymph node strips showed stronger Annexin A8 signals than the post-lymph node strips (Fig. 1E).

Annexin A8 mRNA in cultured human basal and luminal cells. Our whole mammary gland studies did not reveal whether Annexin A8 was expressed in the luminal or basal cells. Recent microarray studies showed that Annexin A8 was more strongly transcribed in several human basal cell–derived cell lines compared with those derived from luminal or endothelial cells. To confirm Annexin A8 as a basal cell marker, we did semiquantitative RT-PCR on total RNA from fluorescence-activated cell sorting–selected and cultured human luminal and basal cells. Our results revealed that although Annexin A8 was expressed more strongly in the basal cells, its transcript could also be found in the luminal cell fraction (Fig. 1F). Control experiments for the basal cell marker α-smooth muscle actin (α-SMA) showed that there was no cross-contamination of the luminal cell fraction with basal cells.

Annexin A8 in normal human breast tissue. To test whether the localization of Annexin A8 protein in vivo reflected our RT-PCR results, we did immunocytochemistry on normal human tissue sections derived from reduction mammoplasties. In 21 individual breast samples tested, the luminal epithelium showed a strong but heterogeneous staining pattern, with luminal cells in lobular structures showing 0% to 100% staining (Fig. 2A). Only weak basal cell staining was observed. The luminal staining was both cytoplasmic and nuclear, but there was often a concentration of staining at the apical aspect of the luminal cell. In the terminal and segmental ducts, there was variable weak cytoplasmic staining in the basal cells.

Changes in Annexin A8 cellular expression from luminal to basal in hyperplastic lesions and in situ carcinomas. The in vivo expression of Annexin A8 in the normal luminal cells was not reflected in the isolated cells in vitro. This raised the possibility of an in vitro modulation of expression. We therefore decided to assess potential change in expression in vivo in a range of benign and malignant breast diseases. Within the spectrum of benign diseases studied, areas of adenosion showed strong basal cell staining. In areas of hyperplasia of usual type, the intraluminal proliferation showed heterogeneous staining (Fig. 2B). In some cases of benign intraluminal proliferation, the cells were all negative with strong expression in the basal layer. In atypical ductal hyperplasia, the atypical cells were all negative, but the basal cells were positive (Fig. 2C). Of the 20 cases of DCIS examined, one grade 2 carcinoma showed granular cytoplasmic positivity. One of 13 cases of lobular carcinoma in situ showed expression of Annexin A8 in the tumor cells. All other cases of DCIS and lobular carcinoma in situ were negative (Fig. 2D).

Annexin A8 and CK5 in infiltrating carcinomas and BRCA1-related breast tumors. In view of the association of Annexin A8 and the basal cell–like cluster, we stained the infiltrating carcinomas for Annexin A8 and CK5. All tumors, with the exception of one medullary carcinoma, were negative. The single medullary carcinoma was positive for both Annexin A8 and CK5.

Following up on the association of medullary carcinoma with BRCA1 tumor phenotype and a high incidence of CK5/6 positivity in BRCA1 tumors, we examined a small cohort of BRCA1 tumors. In microarray studies, BRCA1-related breast tumors clustered together with the basal cell–like subtype, with Annexin A8 and CK5 closely linked. In our study, 12 of 14 tumors (85.7%) from BRCA1 carriers were positive for Annexin A8 and coexpressed CK5 (Table 1; Fig. 2D). The other two tumors were negative for either protein.

Tissue array analysis of Annexin A8 protein expression in invasive breast carcinoma. Because Annexin A8 is a component of a gene cluster that correlates with poor prognosis, it is important to know the relative contribution made by the individual genes to this new prognostic index. We therefore stained 1,631 breast carcinomas that had been put into tissue arrays; 441 cases showed weak to strong staining, with weak staining tending to be diffuse (93.9%) and the strong tending to be nuclear (67.5%; P < 0.0001). Annexin A8 expression was significantly associated with tumor grade (Table 2), with strongly positive tumors more likely to be grade 3 (67.5% versus 30.7% grade 1 and 37.2% grade 2; P < 0.0001). Annexin A8 expression was strongly associated with CK5, with 96% of the tumors negative or weak for Annexin A8 also negative for CK5. Annexin A8 expression was also associated with negative ER status (P < 0.0001) and positive epidermal growth factor receptor status (P < 0.0001). There was a higher percentage of medullary tumors among the strongly positive (P < 0.0001), whereas a lobular histology tended to be associated more with negative Annexin A8 staining (P = 0.007). There was no association with tumor size, nodal status, or age. We further compared Annexin A8 staining with the individual components of tumor grade (tubule formation, pleomorphism, and mitoses). Although there was a significant difference in tubule formation (P = 0.03), there was no significant correlation (P = 0.42). However, pleomorphism and mitoses showed the
same statistically significant association as overall grade ($P < 0.0001$) and both were statistically significant in tests for correlation ($P < 0.0001$).

In our cohort, the standard prognostic factors were highly significant in the univariate analyses (grade, $P < 0.0001$; tumor size, $P < 0.0001$; nodal status, $P < 0.0001$; age, $P < 0.0001$; ER status, $P < 0.0001$). CK5 was also significantly associated with poor prognosis in our cohort ($P = 0.03$). Looking at the survival outcome of the three Annexin A8 groups (negative, weak, and strong staining; Fig. 3), we found a significant overall difference ($P = 0.03$). Pairwise comparisons revealed that the strongly positive group had a significantly worse outcome than the negative (log-rank pairwise, $P = 0.03$) and weakly positive (log-rank pairwise, $P = 0.009$).

To determine whether Annexin A8 was an independent prognostic factor, we did a multivariate analysis, including Annexin A8 group, histologic grade, tumor size, nodal status, age group, and ER status as defined in Table 2. For the model, we used two indicator variables with negative as the reference category (Table 3). The unadjusted model was significant ($P = 0.03$); however, when adjusted for histologic grade, the differences were no longer significant, indicating that Annexin A8 was not independent of grade. Likewise, when adjusted for CK5, which was also significant unadjusted ($P = 0.03$), neither Annexin A8 nor CK5 were significant, indicating they are highly correlated and therefore CK5 was not included in the final model. When all the other factors were included in the model, each factor had a significant effect on survival except Annexin A8, indicating that grade, tumor size, nodal status, ER status, and age group were all independent prognostic factors, but Annexin A8 was not.

To assess whether there was any evidence of a difference within nodal status subpopulations, we did the same analyses on lymph node–positive and lymph node–negative cohorts separately. Both subpopulations behaved in the same way as the overall cohort (data not shown).

### Discussion

Annexin A8 is a poorly described member of the annexin family found in the corneal endothelium (24), lung, kidney, skin, and liver extracts, using ELISA or immunocytochemistry (25). In bovine endochondral ossification, it is a marker of chondrocyte differentiation (26). Annexin A8 RNA was also found during mouse palatogenesis (5) and in an acute promyelocytic leukemia cell line (13).

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**Table 2. Baseline characteristics according to Annexin A8 group**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Annexin A8</th>
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<tbody>
<tr>
<td></td>
<td>Negative, $n$ (%)</td>
</tr>
<tr>
<td>BRE grade 1</td>
<td>299 (25.1)</td>
</tr>
<tr>
<td>BRE grade 2</td>
<td>526 (44.2)</td>
</tr>
<tr>
<td>BRE grade 3</td>
<td>365 (30.7)</td>
</tr>
<tr>
<td>Tumor size (cm) ≤2</td>
<td>412 (35.5)</td>
</tr>
<tr>
<td>Tumor size (cm) &gt;2</td>
<td>750 (64.5)</td>
</tr>
<tr>
<td>Nodal status negative</td>
<td>472 (48.3)</td>
</tr>
<tr>
<td>Nodal status positive</td>
<td>506 (51.7)</td>
</tr>
<tr>
<td>Age (y) &lt;55</td>
<td>352 (29.6)</td>
</tr>
<tr>
<td>Age (y) ≥55</td>
<td>836 (70.4)</td>
</tr>
<tr>
<td>ER status negative</td>
<td>220 (19.1)</td>
</tr>
<tr>
<td>ER status positive</td>
<td>930 (80.9)</td>
</tr>
<tr>
<td>EGFR status negative</td>
<td>1,063 (94.8)</td>
</tr>
<tr>
<td>EGFR status weak/moderate</td>
<td>31 (2.8)</td>
</tr>
<tr>
<td>EGFR status strong</td>
<td>27 (2.4)</td>
</tr>
<tr>
<td>CK5 status negative</td>
<td>1,105 (97.2)</td>
</tr>
<tr>
<td>CK5 status positive</td>
<td>32 (2.8)</td>
</tr>
<tr>
<td>CK5 status missing*</td>
<td>53 (2.8)</td>
</tr>
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(Continued on the following page)
We have now shown that Annexin A8 is up-regulated at the onset of mouse mammary gland involution and that during pubertal morphogenesis expression was only detectable in the resting ductal epithelium. The latter finding is in accordance with the result that Annexin A8 is more highly expressed in human breast epithelial cells grown under differentiating conditions than in the same cells grown as undifferentiated mammospheres (27). However, Annexin A8 expression during times of alveolar differentiation (late pregnancy and lactation) was not detectable in the mouse mammary gland, making it unlikely that Annexin A8 is a general differentiation factor.

Annexins A1, A2, and A4 have been reported in cytoplasmic and nuclear fractions of bovine mammary glands (28, 29). Like Annexin A8, Annexin A1 and Annexin A2 expression also decreased during lactation in cows and rats (29, 30) and enforced weaning led to a strong increase in the level of Annexin A1 mRNA and protein. In our microarray experiment, Annexin A1 and Annexin A2 behaved similarly to each other, with both genes being down-regulated during lactation and returning to pre-lactation levels after enforced weaning (data not shown). In addition, Annexin A1 and Annexin A2 showed similar expression levels in pubertal ducts and terminal end buds (data not shown). In contrast, Annexin A8 mRNA could only be detected in the ducts and was the only annexin with an increase to higher than pre-lactation levels during early involution. However, Annexin A8’s physiologic function is still unclear and can only be speculated upon.

One possibility is based on the potential anti-inflammatory activity of many annexins, by regulating phospholipase A2 activity (4, 31). Our group and others have recently shown that mouse mammary gland involution is associated with an immune response and suppressed inflammation (19, 32), and it is therefore possible that Annexin A8 might contribute to the anti-inflammatory response. Many annexins have also been associated with phagosomes (2, 33), and because luminal alveolar cells are the main phagocytes during early involution (34, 35), it could be speculated that Annexin A8 might be involved in the phagocytosis of neighboring apoptotic cells. Whether Annexin A8 could be directly involved in the apoptosis that occurs during involution is not known. In human BRCA1-associated breast carcinoma, Annexin A8 expression did not correlate with apoptosis; however, it is

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**Table 2. Baseline characteristics according to Annexin A8 group (Cont’d)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Annexin A8</th>
</tr>
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<tr>
<td></td>
<td>Negative, n (%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Adenoid cystic</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Apocrine</td>
<td>9 (0.76)</td>
</tr>
<tr>
<td>Atypical medullary</td>
<td>2 (0.17)</td>
</tr>
<tr>
<td>Carcinosarcoma</td>
<td>1 (0.08)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>11 (0.92)</td>
</tr>
<tr>
<td>Cribriform</td>
<td>34 (2.86)</td>
</tr>
<tr>
<td>Ductal</td>
<td>854 (71.76)</td>
</tr>
<tr>
<td>Ductal</td>
<td>5 (6.02)</td>
</tr>
<tr>
<td>Metaplastic</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>30 (2.52)</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Papillary</td>
<td>14 (1.18)</td>
</tr>
<tr>
<td>Nonmedullary</td>
<td>1,169 (98.24)</td>
</tr>
<tr>
<td>Signet ring</td>
<td>1 (0.08)</td>
</tr>
<tr>
<td>Tubular</td>
<td>28 (2.35)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
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<tr>
<td>Lobular</td>
<td>21 (1.76)</td>
</tr>
<tr>
<td>Nonlobular</td>
<td>1,190 (100.0)</td>
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</table>

Abbreviations: BRE, Bloom, Richardson, Elston; EGFR, epidermal growth factor receptor.

^1 Cases with missing information are not included in tests for significance and percentages.

^2 *χ²*.
not clear whether the same is true for the involuting mouse mammary gland.

In the human breast, Annexin A8 was expressed in the lobular and ductal luminal epithelium and some basal cells of major ducts and was rarely expressed in tumor cells. In this respect, Annexin A8 behaved similarly to Annexin A1, which is localized in acini and ductal basal cells of the normal human breast but not in tumor tissue (36). However, Annexin A1 was found to bind to CK8 and CK18, two luminal cell marker proteins (37), whereas Annexin A8 RNA expression was linked with the expression of CK5 and CK17 in recent microarray studies (15–17). Protein expression of Annexin A8 and CK5 also correlated in our study of BRCA1-related and spontaneous breast carcinomas. Annexin A8 may therefore also act as an intermediate filament associated protein in the human breast. It is unknown whether the same coexpression between CK5 and Annexin A8 exists in the mouse mammary gland during involution.

Annexin A8, CK5, and CK17 were coexpressed in a basal cell–like subgroup of human breast carcinomas with poor prognosis (15–17). The name refers to a specific expression of these genes in basal epithelial cell lines. Basal cell–like carcinomas have long been of particular interest, because this tumor type is associated with a reduced rate of survival (16, 17, 38–40). In addition, immunocytochemical studies have since shown that CK5 and CK17 are markers for a subgroup of lymph node–negative breast carcinomas with poor clinical outcome (18). The term basal needs clarification and this has been the subject of a recent review (41). In summary, in the breast the term “basal” has acquired two meanings. In one context, it has become synonymous with breast myoepithelium and in the other it defines a specific subpopulation of basal cytokeratin expressing cells that counterintuitively may be found in either a luminal or basal location in normal glands.

Our studies have now shown that Annexin A8 is expressed in the luminal cells of the normal human breast. The difference in Annexin A8 expression might be explained by culturing artifacts, because in our hands, cultured primary basal cells also showed a stronger expression than luminal cells. It has also been shown that cultured primary luminal epithelial cells can convert into basal cells (21), and it also is possible that the cell lines used for the microarray studies arose from Annexin A8–expressing basal cells. However, our cultured luminal cells still expressed Annexin A8 mRNA. Our data on Annexin A8 expression in benign disease and in situ carcinomas also support the view that the protein can be up-regulated in basal cells in vivo. This is further supported by a recent study, describing an extensive change in gene expression in the basal cells during breast cancer progression (42). Therefore, neither Annexin A8 nor CK5 can be seen as reliable in vivo basal (myoepithelial) cell markers.

In our study, Annexin A8 expression was significantly associated with the medullary phenotype and inversely with infiltrating lobular carcinoma. Other special tumor types had a low level of expression. It has been previously reported that medullary carcinomas have a high incidence of positivity for basal cell–like markers (43). We have also recently reported distinct genetic and epigenetic changes in medullary carcinoma, with chromosome copy number changes showing some similarities to BRCA1-associated tumors (44). Our results support the emerging data indicating that BRCA1-associated tumors and medullary carcinomas share morphologic and genetic similarities.

BRCA1-related breast tumors have recently been shown to have a similar RNA expression pattern to the basal-like subtype of breast cancers and to be significantly associated with CK5/6 protein expression (17, 45). Our data support the association of Annexin A8 and CK5 expression and BRCA1-related tumors.

| Table 3. Multivariate analysis of Annexin A8 |
|---|---|---|---|
| **Unadjusted** | **Adjusted** |
| **For grade** | **For CK5 status** | **For grade and others*** |
| **HR (95% CI)** | **P** | **HR (95% CI)** | **P** | **HR (95% CI)** | **P** |
| **Negative** | 1.00 | 1.00 | 1.00 | 1.00 | 0.81 (0.62–1.06) | 0.12 |
| **Weak** | 0.89 (0.71–1.10) | 0.26 | 0.85 (0.68–1.05) | 0.12 | 0.90 (0.72–1.12) | 0.34 |
| **Strong** | 1.46 (1.04–2.04) | 0.03 | 1.05 (0.75–1.48) | 0.76 | 1.36 (0.92–2.01) | 0.12 |

*Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.**

**Tumor size, nodal status, age group, and ER status.**
However, this might be a reflection of a functional association; for example, Annexin A1 is known to coassociate with CK8 and CK18 through its NH2-terminal domain (37). Our data show that Annexin A8 expression plays a significant role in defining the poor prognosis phenotype as defined by the microarray cluster. It will be necessary to confirm these data in an independent patient cohort. These data are however, sufficiently interesting to suggest that analysis of the expression of this protein may provide one component of a panel of markers to define breast cancer subgroups. In addition, if the proteins that define the basal cell–like subgroup are functionally involved in the poor prognosis phenotype, it may be predicted that they might form suitable therapeutic targets. It is therefore important that work is now focused on understanding the biology of this and other proteins that are associated with poor prognosis as well as considering them as markers.

References

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