Expression of the ELAV-Like Protein HuR Is Associated with Higher Tumor Grade and Increased Cyclooxygenase-2 Expression in Human Breast Carcinoma

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ABSTRACT

Purpose: The human ELAV (embryonic lethal abnormal vision)-like protein HuR stabilizes a certain group of cellular mRNAs that contain AU-rich elements in their 3′-untranslated region. Cell culture studies have shown that the mRNA of cyclooxygenase (COX)-2 can be stabilized by HuR.

Experimental Design: To investigate a possible contribution of dysregulation of mRNA stability to the progression of cancer and to overexpression of COX-2, we studied expression of HuR in 208 primary breast carcinomas by immunohistochemistry.

Results: There were two different staining patterns of HuR in tumor tissue of breast carcinomas: nuclear expression was seen in 61% of cases, and an additional cytoplasmic expression was seen in 30% of cases. Expression of HuR was significantly associated with increased COX-2 expression; this association was particularly significant for cytoplasmic HuR expression (P < 0.0005). We further observed a significant association of cytoplasmic (P = 0.002) or nuclear HuR (P = 0.027) expression with increased tumor grade. Only 13% of the grade 1 carcinomas showed cytoplasmic expression of HuR, compared with 46% of the grade 3 carcinomas. There was no significant correlation between HuR expression and other clinicopathological parameters such as histological type, tumor size, or nodal status as well as patient survival.

Conclusions: Our results suggest that overexpression of HuR in tumor tissue may be part of a regulatory pathway that controls the mRNA stability of several important targets in tumor biology, such as COX-2. Based on our results, additional studies are necessary to investigate whether HuR might be a potential target for molecular tumor therapy.

INTRODUCTION

Regulation of gene expression by posttranscriptional modification of mRNA stability and translation is an important mechanism used in the adaptation of cells during inflammatory responses as well as in the control of cellular growth (1). AU-rich elements (AREs) in the 3′-untranslated region of some mRNAs are involved in regulation of degradation of mRNAs and provide an effective way to control protein expression by regulation of mRNA half-life as well as translation. Besides its physiological role in inflammation and cell proliferation, dysregulation of mRNA stability may also be relevant in tumor biology and may lead to abnormal expression of several proteins in malignant tumors. A group of proteins that are able to bind to AREs to control mRNA stability has been identified. Among these proteins, the human family of ELAV-like proteins consists of four members (Hel-N1/HuB, HuC, HuD, and HuR) that are involved in posttranscriptional regulation of mRNA turnover and mRNA stability (see the review in Ref. 2). These human proteins are highly conserved and show homology to the Drosophila nuclear protein ELAV (embryonic lethal abnormal vision). Three of these four proteins (Hel-N1/HuB, HuC, and HuD) are expressed mainly in neuronal tissue. In contrast, the fourth protein, HuR, is expressed in many cell types and stabilizes cellular mRNAs that contain AREs in their 3′-untranslated region. In cell culture studies, HuR is expressed mainly in the cellular nucleus, whereas only small amounts of HuR are present in the cytoplasm. Several studies suggest that these comparably small amounts of cytoplasmic HuR are relevant for its mRNA stabilizing activity and that HuR is a nuclear shuttling protein (3–5).

Breast cancer is the most common malignancy in women. In 2003, 211,000 new cases of breast cancer were estimated to be diagnosed in the United States, and approximately 40,000 patients will die of the disease (6). Accepted prognostic and predictive factors of breast carcinoma include nodal status, tumor size, tumor grade, and, to some extent, patient age (7, 8). We have recently shown that expression of cyclooxygenase (COX)-2 is an additional prognostic factor in human breast cancer (9). COX-2 is the rate-limiting enzyme in the synthesis of prostaglandins and is overexpressed in several types of malignant tumors (see the reviews in Refs. 10 and 11). It has recently been shown that COX-2 mRNA contains an ARE in its 3′-untranslated region that serves as a binding site for HuR, resulting in an increased mRNA half-life (12, 13). Thus, the regulation of overexpression of COX-2 in malignant breast tumors may provide an interesting example for a possible contribution of dysregulation of mRNA stability to the progression of cancer.
Therefore, we investigated the expression and cellular localization of HuR in a cohort of 208 primary human breast carcinomas. The expression pattern of HuR was correlated with the expression of COX-2 and COX-1 in tumor tissue.

PATIENTS AND METHODS

Study Population. Immunohistochemical analysis was performed retrospectively on tissue samples taken for routine diagnostic purposes. Based on the availability of tissue in the archives of the Institute of Pathology of the Charité University Hospital (Berlin, Germany), 208 patients with primary breast carcinoma who were diagnosed between June 1991 and June 1996 and had available follow-up data were identified. Patients with distant metastasis at the time of diagnosis or bilateral breast carcinomas were not included in this study. Data on overall survival were available for all patients; data on disease recurrence were available for 159 of the 208 (76%) patients. Median follow-up time of all patients still alive at the time of analysis was 86 months (range, 5–130 months). Within the follow-up period, 72 patients (34.6%) died. Clinical data on disease-free survival were defined as the time between diagnosis and the first clinical or pathological evidence of locoregional or distant recurrence. Forty-six of the 159 patients (29%) experienced disease recurrence.

Baseline patient characteristics are given in Table 1. The median age of all patients at the time of diagnosis was 60 years (range, 28–88 years). Disease was classified as follows: 167 patients (80%) had ductal carcinoma, 27 patients (13%) had lobular carcinoma; and 14 patients (7%) had other histology. A total of 155 of the 208 (74.5%) patients had been operated on by the same surgeon (K-J. W.). In addition to the carcinomas, 7 samples of benign breast tissue from patients with fibrocystic disease were stained for HuR as well. These samples were not included in the statistical evaluation.

Histopathological Examination. Tissue samples were fixed in 4% neutral buffered formaldehyde, embedded in paraffin, and evaluated using H&E-stained sections. Tumor histology and grade were evaluated at primary diagnosis and extracted from the pathology report. Cases were graded according to the Bloom-Richardson Grading modified by Elston and Ellis (14). Nodal status was assessed at primary diagnosis. For this study, nodal status was reclassified according to the sixth edition of the tumor-node-metastasis (TNM) classification (15).

Immunohistochemistry. For HuR immunohistochemistry, we used monoclonal antihuman HuR antibody 3A2 (1:250; Santa Cruz Biotechnology, Santa Cruz, CA) with antigen retrieval in citrate buffer in a pressure cooker for 5 min. Slides were incubated with a biotinylated antimouse secondary antibody and the multilink biotin-streptavidin-amplified detection system (Biogenex, San Ramon, CA). Staining was visualized using a fast-red chromogen system (Sigma, St. Louis, MO).

The intensity of the cytoplasmic and nuclear immunostaining in tumor cells was evaluated independently by two investigators (W. W. and C. D.), who were blinded to patient outcome. Cases for which both investigators disagreed on the immunoreactive score were discussed using a multiheaded microscope until consensus was achieved. The cytoplasmic and nuclear staining patterns of HuR were evaluated separately, each according to the percentage of positive cells and the intensity of staining. For each case, one complete histological section was evaluated. The percentage of positive cells was scored as follows: 0 (0% positive cells); 1 (<10% positive cells); 2 (10–50% positive cells); 3 (50–80% positive cells); 4 (>80% positive cells). The staining intensity was scored as follows: 0 (negative staining); 1 (weak staining); 2 (moderate staining); and 3 (strong staining). For the immunoreactive score, the percentage of positive cells and staining intensity were multiplied, resulting in a value between 0 and 12 (16). To separate cases with a low or strong expression of cytoplasmic or nuclear HuR, we combined cases with an immunoreactive score of 0–6 in one group with negative to low HuR expression (HuR-negative group), whereas cases with an immunoreactive score of 7–12 were combined into a HuR-positive group. To exclude the possibility that cytoplasmic HuR expression might be the result of a formalin fixation artifact, we controlled expression of HuR in stromal cells and in nonneoplastic epithelium as well. The cytoplasmic expression of HuR was restricted to the tumor cells and was not found in adjacent stromal cells or in normal epithelium. Data on expression of COX-2 and COX-1 had been obtained in a previous study for all tumors (9).

**Statistical Analysis.** The statistical significance of the correlation between expression of HuR and several clinicopath-
ological parameters as well as COX-1 or COX-2 was assessed by Fisher’s exact test, $\chi^2$ test, or $\chi^2$ test for trends, as indicated. The probability of overall survival as a function of time was determined by the Kaplan-Meier method and the log-rank test. Generally, $P$ values of $<0.05$ were considered significant. For statistical evaluation, SPSS software version 10.0 was used.

RESULTS

Expression of HuR in Human Breast Carcinoma. Because HuR has been described as a nuclear shuttling protein whose cellular distribution may be important for its interaction with AREs and its mRNA stabilizing activity, we investigated the nuclear and cytoplasmic expression of HuR independently, generating a separate immunoreactive score for each staining pattern. Thus, using immunohistochemical examination of HuR in the invasive breast carcinomas, two different staining patterns were observed. As shown in Fig. 1 and Table 1, nuclear expression of HuR was present in 128 of the 208 cases (61%). In addition, we found cytoplasmic expression of HuR in a subset of 63 cases (30%). In 47 cases (37%), a positive nuclear HuR immunoreactivity and a positive cytoplasmic HuR immunoreactivity were observed. There was a significant positive correlation between nuclear and cytoplasmic immunoreactivity ($P = 0.008$, Fisher’s exact test). In the cases with cytoplasmic expression of HuR, this expression pattern was restricted to the tumor cells, whereas stromal cells and adjacent nonneoplastic tissue did not show cytoplasmic expression of HuR (Fig. 1F).

In addition to the invasive breast carcinomas, expression of HuR was investigated in the ductal carcinoma in situ that was found adjacent to the invasive carcinomas in 39 cases. In these cases, the in situ carcinomas showed a nuclear and cytoplasmic HuR staining pattern that was comparable with the adjacent
invasive carcinoma. To investigate the expression pattern in
nonmalignant breast lesions, tissue from seven cases with fibro-
cystic disease was studied. Of these seven cases, none showed a
positive cytoplasmic HuR immunoreactivity, whereas in four
cases (57%), a positive nuclear immunoreactivity was found.

Correlation with COX-2 and COX-1 Expression. Because HuR has
been described as a protein that is responsible for
stabilization of COX-2 mRNA, we investigated the correlation
between increased cytoplasmic or nuclear HuR expression and
COX-1 or COX-2 immunoreactivity. As shown in Table 2 and
Fig. 2, an increased cytoplasmic immunoreactivity was signifi-
cantly associated with an increased COX-2 expression. Whereas
only 22% of the COX-2 negative cases showed cytoplasmic
HuR immunoreactivity, this percentage was increased to 48%
for the COX-2-positive cases \( (P = 0.0005) \). In contrast, cyto-
plasmic HuR was not significantly correlated with COX-1 ex-
pression. The group of 47 cases that were positive for both
cytoplasmic and nuclear HuR expression was significantly as-
sociated with increased COX-2 expression \( (P = 0.0005) \; \text{data}
not shown).

As shown in Table 3, nuclear expression of HuR in inva-
sive breast carcinomas was correlated with COX-2 expression
as well; however, this correlation was not as strong as that for
cytoplasmic HuR \( (P = 0.033) \). Interestingly, the nuclear
expression of HuR was highly significantly correlated with an
increased expression of the COX-1 isoform in tumor tissue \( (P <
0.0005) \; \text{Table 3}.\)

Correlation with Clinicopathological Parameters. For
evaluation of breast carcinomas, several clinicopathological pa-
rameters are used, such as histological type, tumor size, the
presence of lymph node metastases, and the degree of tumor
differentiation (tumor grade). We investigated the correlation
of cytoplasmic or nuclear HuR expression with these established
clinicopathological tumor parameters (Tables 2 and 3). In the
invasive breast carcinomas, we observed a significant associa-
tion of cytoplasmic HuR expression with increased tumor grade.
Whereas only 13% of grade 1 carcinomas showed cytoplasmic
expression of HuR, the percentage was increased to 46% for
grade 3 carcinomas \( (P = 0.002; \text{Fig. 2}) \). A similar associa-
tion was observed between nuclear expression of HuR and histolog-

![Table 2](https://example.com/table2)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cytoplasmic HuR negative (score, 0–6)</th>
<th>Cytoplasmic HuR positive (score, 7–12)</th>
<th>( P )</th>
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</thead>
<tbody>
<tr>
<td>All patients</td>
<td>145 (69.7%)</td>
<td>63 (30.3%)</td>
<td>&lt;0.0005*</td>
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<tr>
<td>COX-2 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>109 (78.4%)</td>
<td>30 (21.6%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>36 (52.2%)</td>
<td>33 (47.8%)</td>
<td></td>
</tr>
<tr>
<td>COX-1 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>82 (71.9%)</td>
<td>32 (28.1%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>63 (67.0%)</td>
<td>31 (33.0%)</td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal carcinoma</td>
<td>112 (67.1%)</td>
<td>55 (32.9%)</td>
<td></td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>23 (85.2%)</td>
<td>4 (14.8%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10 (71.4%)</td>
<td>4 (28.6%)</td>
<td></td>
</tr>
<tr>
<td>Tumor size (mm)</td>
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<td></td>
</tr>
<tr>
<td>( \leq 20 )</td>
<td></td>
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<td></td>
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<tr>
<td>All patients</td>
<td>97 (71.9%)</td>
<td>38 (28.1%)</td>
<td></td>
</tr>
<tr>
<td>( &gt; 20 )</td>
<td>48 (65.8%)</td>
<td>25 (34.2%)</td>
<td></td>
</tr>
<tr>
<td>Nodal status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pN0/pN1mic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>78 (72.2%)</td>
<td>30 (27.8%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>28 (70%)</td>
<td>12 (30%)</td>
<td></td>
</tr>
<tr>
<td>pN2a</td>
<td>21 (72.4%)</td>
<td>8 (27.6%)</td>
<td></td>
</tr>
<tr>
<td>pN3a</td>
<td>18 (58.1%)</td>
<td>13 (41.9%)</td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
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<tr>
<td>G1</td>
<td>47 (87.0%)</td>
<td>7 (13.0%)</td>
<td>0.002†</td>
</tr>
<tr>
<td>G2</td>
<td>71 (67.6%)</td>
<td>34 (32.4%)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>24 (54.5%)</td>
<td>20 (45.5%)</td>
<td></td>
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</tbody>
</table>

Abbreviation: n.s., not significant.
* Fisher’s test.
† \( \chi^2 \) test.

![Fig. 2](https://example.com/fig2)

**Fig. 2** Association between cytoplasmic HuR expression and increased
COX-2 expression in human breast carcinoma tissue \( (P < 0.0005; \text{A}) \). Positive correlation between cytoplasmic \( (P = 0.002; \text{B}) \) or nuclear
\( (P = 0.027; \text{C}) \) expression of HuR and histological tumor grade.
HuR Expression in Breast Carcinoma

In this study, we investigated the expression pattern and prognostic role of the human ELAV-like protein HuR in human breast carcinoma. We detected an increased expression of HuR in the subgroup of poorly differentiated and rapidly growing carcinomas. This suggests that an increased expression of HuR as an indicator of dysregulation of mRNA stability may be involved in the regulation of tumor growth of breast carcinomas.

Investigating COX-2 as a potential target of the mRNA stabilizing activity of HuR, we observed a significant correlation between increased COX-2 expression in tumor tissue and HuR immunoreactivity. This correlation was particularly significant for the cytoplasmic expression of HuR.

To our knowledge, this is the first study investigating the expression of HuR in a large cohort of primary human breast carcinomas. In a recent study, Lopez de Silanes et al. (17) have described HuR expression in several types of tumors and found an increased cytoplasmic HuR expression in five of seven breast carcinomas, which is consistent with our results. Functional data from several in vitro studies suggest that HuR might be an important regulatory factor in breast cancer cell lines. Sengupta et al. (13) have shown that HuR regulates the expression of COX-2 in MDA-MB-231 human breast cancer cells. In addition, HuR is involved in regulation of urokinase-type plasminogen activator (uPA) and uPA receptor in breast cancer cell lines; inhibition of HuR by RNA interference leads to reduced levels of uPA as well as the uPA receptor mRNAs (18). In a mammary epithelial cell line, treatment with taxanes stimulated binding of HuR to COX-2 mRNA, leading to increased mRNA stability (19).

In addition to these in vitro results, our study shows that the association between increased HuR expression and expression of COX-2 is present in human breast carcinomas in vivo, suggesting that HuR is involved in stabilization of COX-2 mRNA and regulation of COX-2 expression in primary human breast carcinomas as well. This association was particularly strong for cases with cytoplasmic expression of HuR, supporting the hypothesis derived from cell culture studies that the mRNA stabilizing activity of HuR is linked to its presence in the cytoplasm. Whereas regulation of mRNA stability seems to be the most important regulatory step for COX-2 expression, several studies have described other mechanisms such as transcriptional control or hypermethylation (20) to be involved in the regulation of COX-2 expression.

### Table 3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nuclear HuR expression</th>
<th>Nuclear HuR expression</th>
<th>Nuclear HuR expression</th>
<th>Nuclear HuR expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
<td>Score (0–6)</td>
<td>Score (7–12)</td>
<td>Score (13–18)</td>
</tr>
<tr>
<td>COX-2 expression</td>
<td>208 (100%)</td>
<td>128 (61.5%)</td>
<td>70 (35.7%)</td>
<td>5 (2.5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>139 (100%)</td>
<td>79 (56.8%)</td>
<td>45 (32.5%)</td>
<td>5 (3.8%)</td>
</tr>
<tr>
<td>Positive</td>
<td>69 (100%)</td>
<td>49 (71.0%)</td>
<td>18 (26.9%)</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>COX-1 expression</td>
<td>114 (100%)</td>
<td>57 (50.0%)</td>
<td>57 (50.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>94 (100%)</td>
<td>71 (75.5%)</td>
<td>23 (24.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Positive</td>
<td>176 (100%)</td>
<td>105 (62.9%)</td>
<td>71 (40.7%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Histological type</td>
<td>167 (100%)</td>
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<td>62 (37.1%)</td>
<td>0 (0.0%)</td>
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<tr>
<td>Ductal carcinoma</td>
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<td>15 (55.6%)</td>
<td>12 (44.4%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>14 (100%)</td>
<td>8 (57.1%)</td>
<td>6 (42.9%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Other</td>
<td>135 (100%)</td>
<td>80 (59.3%)</td>
<td>55 (40.7%)</td>
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<tr>
<td>Tumor size (mm)</td>
<td>135 (100%)</td>
<td>92 (67.7%)</td>
<td>48 (34.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>≤20</td>
<td>135 (100%)</td>
<td>92 (67.7%)</td>
<td>48 (34.5%)</td>
<td>0 (0.0%)</td>
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<tr>
<td>&gt;20</td>
<td>73 (100%)</td>
<td>48 (65.8%)</td>
<td>34 (23.3%)</td>
<td>0 (0.0%)</td>
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<tr>
<td>Nodal status</td>
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<td>[n.s.]^†</td>
<td>[n.s.]^†</td>
<td>[n.s.]^†</td>
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<tr>
<td>pN0/pN1mic</td>
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<td>62 (57.4%)</td>
<td>46 (42.6%)</td>
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</tr>
<tr>
<td>pN1a</td>
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<td>68 (64.8%)</td>
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<tr>
<td>pN2a</td>
<td>54 (100%)</td>
<td>31 (57.4%)</td>
<td>25 (46.3%)</td>
<td>3 (5.6%)</td>
</tr>
<tr>
<td>pN3a</td>
<td>14 (100%)</td>
<td>8 (57.1%)</td>
<td>6 (42.9%)</td>
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</tr>
<tr>
<td>Histological grade</td>
<td>[G3]</td>
<td>[G2]</td>
<td>[G1]</td>
<td>[G0]</td>
</tr>
<tr>
<td>Positive</td>
<td>63 (100%)</td>
<td>25 (40.0%)</td>
<td>38 (60.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>128 (100%)</td>
<td>80 (62.5%)</td>
<td>47 (37.5%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Abbreviation: n.s., not significant.
* Fisher’s test.
† χ² test.

### Table 4

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
<th>No. of events</th>
<th>Median survival time (mo)</th>
<th>Log-rank P</th>
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<tr>
<td>Cytoplasmic HuR expression</td>
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<td></td>
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</tr>
<tr>
<td>Negative</td>
<td>145</td>
<td>47</td>
<td>122.7 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>63</td>
<td>25</td>
<td>119.8 ± 8.5</td>
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<td>Nuclear HuR expression</td>
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<tr>
<td>Negative</td>
<td>80</td>
<td>29</td>
<td>122.2 ± 8.8</td>
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<tr>
<td>Positive</td>
<td>128</td>
<td>43</td>
<td>122.7 ± 6.1</td>
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<tr>
<td>Tumor size (mm)</td>
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<tr>
<td>≤20</td>
<td>135</td>
<td>33</td>
<td>Not reached</td>
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<tr>
<td>&gt;20</td>
<td>73</td>
<td>39</td>
<td>93.1 ± 13.1</td>
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<td>44.0 ± 11.4</td>
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<td>G1</td>
<td>54</td>
<td>11</td>
<td>Not reached</td>
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<tr>
<td>G2</td>
<td>105</td>
<td>37</td>
<td>122.2 ± 2.8</td>
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<tr>
<td>G3</td>
<td>44</td>
<td>23</td>
<td>59.7 ± 19.1</td>
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Univariate survival analysis (Kaplan-Meier): median overall survival time of all patients according to cytoplasmic or nuclear HuR expression and tumor size, nodal status, and histological grade.
COX-2 expression as well. We believe that the finding that there are cases with a negativity for COX-2 despite cytoplasmic HuR positivity may best be explained by these additional regulatory mechanisms for COX-2 expression.

We did not observe an association of cytoplasmic HuR expression with COX-1; however, the nuclear expression of HuR was highly significantly linked to increased levels of COX-1 ($P < 0.0005$). Thus, changes in the cellular distribution of HuR may affect not only COX-2 but also COX-1 expression in the cells. Interestingly, an up-regulation of HuR has been shown in several other tumors as well, e.g., brain tumors (21), ovarian carcinomas (22, 23), and colon carcinomas (17), indicating that a dysregulation of mRNA stability may be involved in the malignant phenotype in these tumors as well. In addition to COX-2, several other cellular mRNA targets for HuR have been reported [for example, the mRNAs of the angiogenic factor vascular endothelial growth factor; the inflammatory cytokines interleukin 8, interleukin 6, and tumor necrosis factor α (21); and cell cycle-regulatory proteins such as cyclin A and cyclin B1 (24)]. In addition, the protein kinase C substrate MARCKS (25), the proto-oncogene c-fos (5), and uPA and uPA receptor have been described as targets of HuR (18). Although it is not known which of the potential targets are relevant in human tumors in vivo, the present data suggest that HuR might serve as a central step in the control of the expression of proteins that are involved in angiogenesis, tumor-associated inflammation, and cellular growth. Expression of HuR might be an important step in adaptation of tumors to the tumor microenvironment and in regulation of the tumor-host interaction.

Because the ability of HuR to shuttle between the nucleus and the cytoplasm is important for its mRNA stabilizing function, several mechanisms for control of the cellular localization of HuR have been identified. The translocation of HuR from the nucleus to the cytoplasm is inhibited by active AMP-activated kinase (26). AMP-activated kinase has been termed the fuel gauge of the cell and is activated by low energy levels. As an additional mechanism, Brennan et al. (27) have identified four proteins (SETα, SETβ, pp32, and APRIL) that are able to interact with HuR. Two of these proteins, pp32 and APRIL, interact with the nuclear export factor CRM1. A third possible mechanism involves the activation of p38MAPK that leads to phosphorylation of mitogen-activated protein kinase-activated protein kinase 2. It has been shown that mitogen-activated protein kinase-activated protein kinase 2 increased cytoplasmic translocation of HuR and the stability of ARE-containing mRNAs (19). Additional studies are needed to investigate which of these regulatory pathways may be relevant for the abnormal cytoplasmic overexpression of HuR observed in breast carcinomas.

Auto-antibodies against Hu antigens are involved in the pathogenesis of paraneoplastic neuronal disorders in tumor patients. Interestingly, the immune response leading to paraneoplastic neuronal disorders is also active against the tumor cells, which is supported by the clinical observation that tumors of paraneoplastic neuronal disorder patients usually stay small, and patient mortality is due mainly to neuronal degeneration, not to cancer growth. It has been pointed out that these observations could be viewed as a biological validation of ELAV/Hu proteins as potential new targets for therapeutic approaches (28). Due to its overexpression in several types of malignant tumors and its interesting biological function, HuR protein may be a promising target for a molecular tumor therapy that would interfere with the expression pattern of several proteins that are important for tumor-associated inflammation, angiogenesis, and tumor proliferation. Recently, a RNA interference-mediated strategy for inhibition of HuR has been described (29).

In conclusion, the cytoplasmic expression of HuR may be part of a regulatory pathway that controls the mRNA stability of several biologically important tumor targets. One of these targets is COX-2. Other targets may be cell cycle-regulatory proteins, vascular endothelial growth factor, and inflammatory cytokines. Based on our results, additional studies are necessary to investigate the regulatory network controlled by HuR and to determine whether HuR might be a potential target for a molecular tumor therapy.

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**Fig. 3** Univariate survival analysis investigating the impact of cytoplasmic (A) or nuclear (B) expression of HuR on overall survival of breast carcinoma patients (dashed lines, HuR negative; solid lines, HuR positive).
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Expression of the ELAV-Like Protein HuR Is Associated with Higher Tumor Grade and Increased Cyclooxygenase-2 Expression in Human Breast Carcinoma

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