ORIGINAL PAPER

Relevance of cellular and serum carbonic anhydrase IX in primary breast cancer

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Abstract

Background   Carbonic anhydrase IX (CAIX) is involved in pH homeostasis, growth and survival of tumor cells. Besides the membranous form of CAIX, a soluble form is detectable in serum (s-CAIX). Overexpression of CAIX in tumors offers the opportunity for therapeutic strategies such as CAIX targeting antibodies. The aim of this study was to examine the relationships of CAIX mRNA expression and s-CAIX levels with clinicopathological parameters and survival of patients with primary breast cancer.

Methods   Tumor tissue of 169 primary breast cancer patients was analyzed for RNA expression by microarray analysis (Affymetrix HG-U133A). Concentration of s-CAIX was determined by ELISA in blood samples of 140 patients.

Results   In tumor tissue, CAIX mRNA signal intensities (MAS5 values) ranged from 34 to 2,513. Higher CAIX expression was associated with younger age ($</\geq50$ years $p = 0.040$), negative hormone receptors (estrogen receptor $p = 0.004$; progesterone receptor $p = 0.022$) and positive nodal status ($p = 0.001$) as well as with shorter disease-free survival ($p = 0.031$). Concentrations of s-CAIX ranged from 56 to 1,500 pg/ml. There was no correlation between s-CAIX and CAIX mRNA levels as well as clinicopathological characteristics or outcome.

Conclusion   In contrast to reported immunohistochemical studies, we performed RNA-based tissue analyses of CAIX expression and, for the first time, analyzed CAIX serum levels in primary breast cancer. The correlations between CAIX RNA expression and prognostic factors and patient outcome support a biologic role of CAIX in early breast cancer. A role of s-CAIX in primary breast cancer is not supported by our findings.

Keywords   Carbonic anhydrase IX · RNA expression · Serum isoform · Primary breast cancer

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Background

Hypoxia and acidosis play a role in carcinogenesis, and cellular hypoxia in cancer cells is reported to correlate with poor prognosis and resistance to therapy (Fang et al. 2008; Gatenby et al. 2007; Potter and Harris 2003). Under anaerobic conditions, glycolysis is increased causing an acidotic environment that leads to upregulation of pH-controlling enzymes such as hypoxia-inducible factor (HIF) and related proteins as carbonic anhydrases (Fang et al. 2008; Gatenby et al. 2007).

Carbonic anhydrase IX (CAIX), one of 15 isoforms in humans, is a membranous metalloenzyme that is involved in pH homeostasis (Potter and Harris 2003; Winum et al. 2009a). It catalyzes the conversion of CO_2 to bicarbonate and proton and thus is involved in pH control. Besides this function, CAIX has been implicated in cell-adhesion, growth and survival of tumor cells. It was shown that inhibition of CAIX was correlated with reduced growth and survival of cancer cells (Robertson et al. 2004; Potter and Harris 2004; Zavada et al. 1993). CAIX is expressed in some normal tissues such as stomach, gall bladder and duodenum (Ivanov et al. 2001). Contrasting its restricted expression in normal tissue, CAIX is observed to be strongly expressed in a variety of malignant tumor tissues: cervical cancer and clear cell carcinoma of the kidney and, at a lower expression level, in carcinomas of the breast, head and neck, lung and brain tumors (Kaluz et al. 2009). A high level of CAIX expression, based on immunohisto-chemical analyses, is associated with a poor prognosis in some solid cancers (Chia et al. 2001; Loncaster et al. 2001). In contrast, patients with renal cell cancer and high CAIX level show a better survival (Bui et al. 2003).

Overexpression of CAIX offers the opportunity for therapeutic strategies such as CAIX targeting antibodies (WX-G250, Rencarex®) which are evaluated in clinical trials of solid tumors, for example, renal cell cancer, but currently not breast cancer (Winum et al. 2009b; Bleumer et al. 2006; Siebels et al. 2011; Bui et al. 2003). CAIX expression might be a predictive marker for anticancer therapy by CAIX-inhibition. In addition, tumor hypoxia is assumed to be induced by agents targeting angiogenesis, for example, the VEGF-antibody bevacizumab (Bareschino et al. 2011). Therefore, the regulation of CAIX could be also of interest in the context of these approaches.

Besides the transmembrane form of CAIX, there is a soluble form (s-CAIX) that is released by proteolytic cleavage and can be detected in serum (Zavada et al. 2003). Serum is easily accessible and s-CAIX is therefore a potential marker for patient stratification, therapy monitoring and prognosis. Although there is some information about s-CAIX in solid tumors and gynecologic tumors, there is only one report about s-CAIX in patients with metastatic breast cancer and no published study in primary breast cancer (Zavada et al. 2003; Woelber et al. 2010; Kock et al. 2011; Carney 2007; Müller et al. 2011).

Several prognostic markers such as tumor size, nodal status and hormone receptor status are currently used to classify breast cancer patients (Mori et al. 2002). Still, many patients seem to be not optimally treated with conventional therapeutics suggesting that this commonly used stratification does not fully consider the heterogeneity of breast cancer. This is also of high relevance in the context of new therapeutic options in breast cancer and other solid tumors.

So far, reports of CAIX expression were based on immunohistochemical analyses. The aim of this study was therefore to investigate the correlation of clinicopathological factors with both cellular CAIX in tumor tissue, based on RNA analyses, and its soluble form s-CAIX in sera of primary breast cancer patients and the potential role of these markers as targets for therapeutic approaches.

Methods

Patients

Tumor tissue samples of 169 patients with primary breast cancer undergoing surgery in the Department of Gynecology, University Medical Center Hamburg-Eppendorf, Germany, were included in this study. Clinical and histopathologic factors (age, nodal status, hormone receptor status, grading, tumor stage, histological subtype) were obtained by reviewing medical charts and pathologic reports. Detailed patients characteristics are listed in Table 1.

Informed consent for the use of patient data, tumor tissue and serum had been obtained from all patients in accordance with local ethics committee according to the principles of the declaration of Helsinki. Patients were treated with surgery, radiotherapy and systemic treatment according to national guidelines. Most patients received adjuvant endocrine treatment and/or chemotherapy in both RNA and serum group (88 and 89 %, respectively). Neither radiotherapy nor neoadjuvant chemotherapy had been performed prior to surgery and tissue acquisition. Patient selection was based upon availability of tumor tissue and/or serum.

CAIX RNA expression

CAIX RNA expression was quantified by microarray analysis (HG-U133A; Affymetrix, Santa Clara, CA. USA) in 169 tumor tissue samples of patients with primary breast cancer. RNA extraction, cDNA synthesis, labeling and microarray analysis were performed as described (Schroder et al. 2011). Affymetrix expression data were analyzed by using the MAS5.0 algorithm according to the Affymetrix statistical algorithms reference guide, technical report 2001.

For correlation with clinicopathological factors, the cohort was divided into two groups of similar size according to CAIX RNA levels (cutoff = median value 134). For survival analysis, CAIX RNA levels were divided in quartiles (cutoffs 91, 134 and 194).

Quantitative analysis of serum CAIX level

Blood samples of 140 patients with primary breast cancer were obtained before surgery, and serum concentration of

Table 1 Patients characteristics (RNA CAIX $n = 169$; Serum CAIX $n = 140$)

	RNA CAIX	Serum CAIX
Age (years)		
Range	29–85	29–85
Median	56.15	56.15
Age <50 years (n)	52	43
Age ≥50 years (n)	117	97
pT		
pT1	40 (24 %)	42 (30 %)
pT2	112 (66 %)	84 (60 %)
pT3	5 (3 %)	5 (4 %)
pT4	8 (5 %)	7 (5 %)
Unknown	4 (2 %)	2 (1 %)
pN		
pN −	120 (71 %)	98 (70 %)
pN +	47 (28 %)	42 (30 %)
Unknown	2 (1 %)	0
Histological subtype		
Ductal	118 (70 %)	80 (57 %)
Lobular	27 (16 %)	23 (17 %)
Others/unknown	24 (14 %)	37 (26 %)
Grading		
1	12 (7 %)	13 (9 %)
2	66 (39 %)	57 (41 %)
3	85 (50 %)	66 (47 %)
Unknown	6 (4 %)	4 (3 %)
Hormone receptor status		
ER positive	118 (70 %)	114 (81 %)
ER negative	42 (25 %)	20 (14 %)
ER unknown	9 (5 %)	6 (5 %)
PR positive	100 (59 %)	115 (82 %)
PR negative	60 (36 %)	21 (15 %)
PR unknown	9 (5 %)	5 (3 %)
Adjuvant therapy		
Chemotherapy	108 (64 %)	89 (64 %)
Endocrine therapy	95 (56 %)	83 (59 %)
Chemo- and/or endocrine therapy	149 (88 %)	124 (89 %)
Survival (months)		
Disease-free survival (mean)	80	78
Overall survival (mean)	84	84
Follow-up (mean and range; months)	84 (7–169)[a]	87 (10–164)

ER estrogen receptor, *PR* progesterone receptor

[a] $n = 167$

CAIX was determined by a commercially available ELISA (Wilex Inc., Oncogene Science, Cambridge, MA 02140, USA). For comparison, serum samples from 48 female healthy volunteers (mean age 64 years; range 50–81 years) were analyzed. Serum samples and controls were diluted 1:2 with sample diluting buffer (containing bovine serum albumin, mouse IgG, buffer salts, and 0.09 % sodium azide). One hundred microliters of the standard, of diluted control samples and of diluted serum samples were dispensed into the wells of 96-well plate (coated with the monoclonal capture antibody) and incubated for two hours at room temperature on a shaker at 800 rpm. Wells were washed, and 100 μl of the detection antibody (containing biotinylated anti-CAIX antibody) was added. The plates were incubated for 30 min at room temperature, washed and then further incubated with 100 μl of a streptavidin horseradish peroxidase conjugate for 30 min at room temperature. After washing, 100 μl of chromogenic substrate (TMB blue substrate) was added for 30 min at room temperature.

The reaction was stopped with 100 μl of 2.5 N sulfuric acid, and absorbance was read at 450 nm by an automated plate reader (Tecan, Crailsheim, Germany). The CAIX concentration was estimated from the standard curve. Each sample, standard and control, was analyzed in duplicate. Intraassay and interassay variation was less than 10 %.

Statistics

Correlations of CAIX and s-CAIX with clinical and histological parameters were performed by chi-square tests. For this purpose, the cohort was first divided into quartiles according to the CAIX values, which were then compared with respect of age (<50 vs. >/=50 years), stage (pT1 vs. 2 vs. 3/4), grading (G1–2 vs. G3), histological type (ductal vs. lobular vs. others), nodal involvement and estrogen receptor (ER) or progesterone receptor (PR) status. Since the median value turned out to be the optimal cutoff, we then performed chi-square tests with two groups (CAIX expression <median vs. >/=median).

Kaplan–Meier analysis of disease-free and overall survival using the Log-rank tests was performed with four groups (quartiles) since the last quartile differed clearly from the rest (Q1–Q3). S-CAIX serum levels in breast cancer samples and controls were compared by two-sided T test. p values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 20. The study was performed in accordance to the REMARK criteria (Hayes et al. 2006).

Results

Patients

A total of 169 tissue samples and 140 blood samples of patients with early breast cancer were included in this

study. In 76 patients, both RNA data and serum levels of CAIX were available. Median age of patients in both cohorts was 56 years.

Most patients had a pT2 tumor stage (66 % in RNA analysis group and 60 % in serum analysis group) and no axillary lymph node metastasis (71, 70 %, respectively). ER and/or PR status was positive in 70 % in the RNA cohort (ER positive 70 %, PR positive 59 %, respectively) and in 85 % of patients in the serum cohort (ER positive 81 %, PR positive 82 %, respectively). Most patients had received an adjuvant systemic therapy in RNA and serum group (chemotherapy in both cohorts 64 %; endocrine treatment 56 and 59 %, respectively). For patient characteristics, see Table 1.

CAIX RNA expression

For CAIX RNA expression analyses, 169 tissue samples of patients with primary breast cancer were included. CAIX RNA signal intensities ranged from 34 to 2,513 with a mean value of 200.05.

High CAIX RNA expression was associated with younger age. Patients younger than 50 years showed a median RNA intensity of 153, while patients with an age of 50 years and older had a lower median signal intensity of 120 ($p = 0.040$; Table 2).

We could demonstrate a significant correlation of high CAIX RNA levels with negative hormone receptor status (ER $p = 0.004$; PR $p = 0.022$) and with positive nodal status ($p = 0.001$). In addition, weak associations of high CAIX RNA expression with poor differentiation (G1/2 vs. G3 $p = 0.050$) and with a ductal histological type ($p = 0.057$) could be found. Table 2 summarizes the correlation of CAIX expression with clinicopathological factors.

By Kaplan–Meier analysis and log-rank test with four groups (quartiles), high CAIX expression was associated with significantly shorter recurrence-free survival ($p = 0.031$, Fig. 1a). By univariate Cox regression analysis, the probability of recurrence in patients with high CAIX expression (highest quartile Q4) was twofold compared to quartiles Q1–Q3 (hazard ratio 1.94; 95 % CI 1.11–3.38; $p = 0.020$). No correlation with overall survival was observed ($p = 0.577$; Fig. 1b).

The prognostic value of CAIX expression was analyzed by Cox regression analysis including tumor stage, hormone receptor status, nodal status and grading. In multivariate analysis, the prognostic value of CAIX expression for shorter recurrence-free survival lost its statistical significance ($p = 0.139$; not shown).

Table 2 Correlation between CAIX RNA expression and clinicopathological factors ($n = 169$)

|	CAIX expression		p value
	<median	>median	
Age			
<50 years	20	32	0.040
≥50 years	65	52	
pT stage			
1	20	20	0.697
2	57	55	
3–4	5	8	
Nodal status			
pN0	69	51	0.001
pN1	14	33	
Hormone receptor			
ER pos.	67	51	0.004
ER neg.	13	29	
PR pos.	57	43	0.022
PR neg.	23	37	
Grading			
1–2	45	33	0.050
3	36	49	
Histological subtype			
Ductal	58	61	0.057
Lobular	18	8	
Others	7	13	

ER estrogen receptor, *PR* progesterone receptor, *pos.* positive, *neg.* negative

Serum CAIX

A total of 140 blood samples taken before surgery of patients with primary breast cancer were analyzed. Concentrations of s-CAIX ranged from 56 to 1,500 pg/ml (median 296 pg/ml) and did not differ significantly from s-CAIX levels in the control cohort of 48 healthy female volunteers (26–1,133 pg/ml; median 199 pg/ml) (Fig. 2). We could not show a correlation with clinical or histological parameters including age, stage, histological type, grading and hormone receptor status (not shown). Furthermore, there was no significant association between s-CAIX and disease-free survival or overall survival.

Correlation CAIX expression and serum CAIX

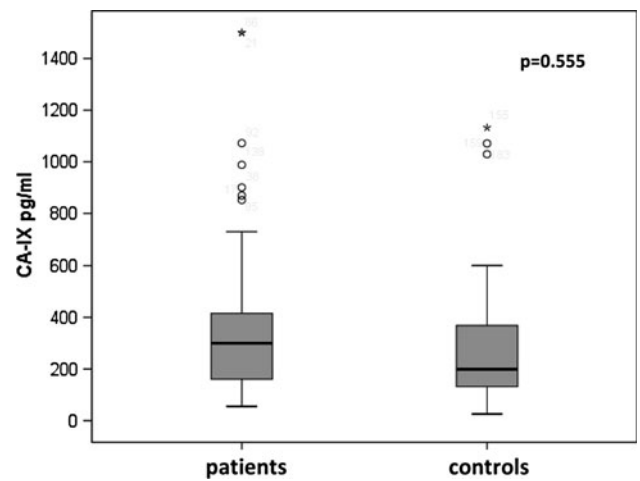In 76 patients, both CAIX RNA and serum level were available.
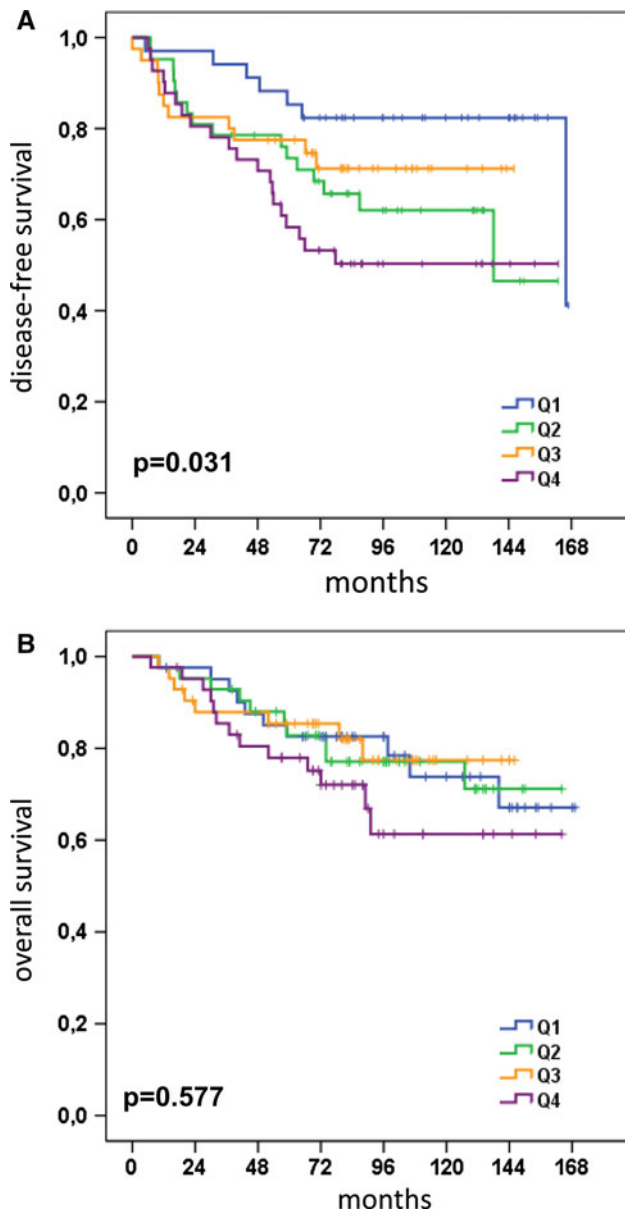
Fig. 1 Kaplan-Meier estimation of **a** disease-free survival and **b** overall survival. Q1–4 = CAIX RNA expression quartile 1–4

Fig. 2 *Box plot* of s-CAIX levels of breast cancer patients and healthy controls. Serum concentrations of s-CAIX ($n = 140$) ranged from 56 to 1,500 pg/ml (median 296 pg/ml) and did not differ largely from s-CAIX levels in the control cohort of 48 healthy female volunteers (26–1,133 pg/ml; median 199 pg/ml) (*T* test $p = 0.555$)

There was no correlation between s-CAIX serum levels and CAIX RNA expression in tumor tissues ($p = 0.332$).

Discussion

Understanding the biology of breast cancer is important for many reasons. One aspect is to estimate prognosis and risk of recurrence in order to stratify patients for treatment. There are several well-established prognostic factors such as tumor size and lymph node metastases as well as proliferation markers associated with more aggressive tumor behavior. Despite this variety of known prognostic factors, not all patients are treated optimally and new prognostic and predictive markers could be helpful to improve stratification for an individualized therapy. In this study, we analyzed CAIX on RNA level in breast cancer to examine its potential biologic relevance.

RNA expression analyses of bulk tumor tissue samples cannot discriminate between expression in carcinoma cells and accompanying normal tissue. However, CAIX expression in normal tissue is highly restricted to specified tissue as, for example, intestinal tissue but not breast tissue (Ivanov et al. 2001; Hussain et al. 2007). In contrast, CAIX is expressed in a variety of malignant tumors, for example, breast cancer (Kaluz et al. 2009). Therefore, detection of CAIX RNA in breast cancer tissue samples might be mostly due to malignant cells and not normal breast cells.

As a membrane protein, CAIX might be an interesting target for treatment. To our knowledge, no reports so far evaluated the aspect of RNA-based tissue analyses and quantitative protein measurement in serum for CAIX. Both approaches are of clinical relevance. The RNA-based tissue analyses could be integrated into multigene analyses performed for prognostic and predictive purposes, and serum is easy to obtain during the course of treatment.

A prognostic relevance of CAIX protein expression was already described in breast cancer. Lou et al. analyzed the expression of CAIX in tumor tissue microarray and could demonstrate that CAIX is detectable by immunohistochemistry in approximately 16 % of patients (Lou et al. 2011). Other reports detected CAIX expression by immunohistochemistry in 26–48 % of breast cancer patients, with a correlation between CAIX expression and poor

prognosis. (Hussain et al. 2007; Chia et al. 2001; Brennan et al. 2006). These results are consistent with our data based on RNA analysis that show a shorter disease-free survival of patients with high CAIX expression. However, in our study, we could not demonstrate a correlation between CAIX RNA expression and overall survival ($p = 0.577$). Brennan et al. analyzed CAIX expression in premenopausal breast cancer patients. Multivariate analysis showed CAIX as an independent prognostic factor for poor survival (Brennan et al. 2006). In our patient cohort, the prognostic value of CAIX expression lost its statistical significance in multivariate analysis. This less pronounced prognostic relevance observed could be explained by the influence of treatment after recurrence and the size of the patient cohort as well as different techniques used for CAIX analyses.

Correlation between shorter survival and high CAIX level might be associated with other aggressive tumor characteristics. Our data show significant relationships between high CAIX RNA level and poor differentiation, young age and negative hormone receptor status which might explain why CAIX lost its prognostic relevance in multivariate analysis. In prior studies, downregulation of ER in hypoxic environment and hypoxia-induced expression of CAIX was described which is consistent with the observed association of high CAIX and negative ER levels (Stoner et al. 2002).

Besides the negative correlation between CAIX mRNA levels and hormone receptor status, we observed a positive correlation to lymph node metastases. Tafreshi and coworkers analyzed CAIX expression in lymph node metastases in breast cancer patients and developed targeted imaging probes by CAIX-specific antibodies to detect sentinel lymph node metastases in a mouse model (Tafreshi et al. 2012). In our present study, only primary tumors were investigated.

CAIX expression is also described in gynecologic tumors, that is, cervical, ovarian and vulvar malignancies. In cervical cancer, high CAIX levels, analyzed by immunohistochemistry, correlate with advanced tumor stage and lymph node metastases (Woelber et al. 2011). In vulvar cancer patients, we observed a shorter disease-free survival in cases with high CAIX expression (Kock et al. 2011). In ovarian cancer, a prognostic relevance was also observed for high CAIX protein expression (Choschzick et al. 2011).

In contrast to CAIX protein expression analyses, studies examining s-CAIX in breast cancer patients are rare. Serum CAIX is easy to measure by blood tests and might not only be a prognostic parameter but also a potential marker of treatment response. Thus, we also examined s-CAIX in our study. Our data in patients without metastases do not point to any correlations with clinical or histological parameters and do not support a role of s-CAIX measurements in clinical routine.

S-CAIX was also analyzed in renal cell cancer patients showing higher values in patients with metastatic tumors than in localized disease. Besides, high serum levels were associated with disease recurrence (Li et al. 2008). Elevated s-CAIX values were also observed in gynecological tumor patients with different results with respect of its potential prognostic role (Woelber et al. 2011; Woelber et al. 2010; Kock et al. 2011).

We previously could show a prognostic relevance of s-CAIX in metastatic breast cancer patients. High levels were correlated with a shorter progression-free survival and overall survival and the presence of circulating tumor cells (Müller et al. 2011). Our study is the first one investigating the role of s-CAIX level in primary breast cancer patients. However, in contrast to metastatic tumors, we could not show a prognostic relevance of s-CAIX in our cohort. A possible explanation for this might be changed tumor characteristics in patients with primary and metastatic breast cancer. In a similar way, the breast cancer associated tumor marker CA 15-3 showed different prognostic relevance in localized and metastatic breast cancer (Duffy et al. 2010). A potential drawback of our study is the incomplete overlap between the cohorts for serum and tissue determination of CAIX. However, it seems very unlikely that the results would differ with a greater overlap.

Despite the fact that we did not observe prognostic relevance for s-CAIX, this marker might be of interest in the context of therapeutic approaches directly targeting the CAIX protein or of strategies targeting angiogenesis thereby affecting the oxygenation of the tumors, for example, the VEGF-antibody bevacizumab. However, our cohort of patients did not receive targeted treatment, and therefore, we cannot draw any conclusions concerning this issue.

Conclusion

In contrast to reported studies, we performed RNA-based tissue analyses of CAIX expression and, for the first time, we analyzed CAIX serum levels in primary breast cancer. The correlation between CAIX RNA expression in tumor tissue and prognostic markers such as hormone receptor status, age and lymph node metastases as well as with shorter disease-free survival support a biologic role of CAIX in early breast cancer. The role of s-CAIX in primary breast cancer as predictive marker in the context of new treatment approaches remains uncertain.

References

Bareschino MA, Schettino C, Colantuoni G, Rossi E, Rossi A, Maione P, Ciardiello F, Gridelli C (2011) The role of antiangiogenetic agents in the treatment of breast cancer. Curr Med Chem 18(33):5022–5032

Bleumer I, Oosterwijk E, Oosterwijk-Wakka JC, Voller MC, Melchior S, Warnaar SO, Mala C, Beck J, Mulders PF (2006) A clinical trial with chimeric monoclonal antibody WX-G250 and low dose interleukin-2 pulsing scheme for advanced renal cell carcinoma. J Urol 175(1):57–62. doi:10.1016/S0022-5347(05)00040-6

Brennan DJ, Jirstrom K, Kronblad A, Millikan RC, Landberg G, Duffy MJ, Ryden L, Gallagher WM, O'Brien SL (2006) CA IX is an independent prognostic marker in premenopausal breast cancer patients with one to three positive lymph nodes and a putative marker of radiation resistance. Clin Cancer Res 12(21):6421–6431. doi:10.1158/1078-0432.CCR-06-0480

Bui MH, Seligson D, Han KR, Pantuck AJ, Dorey FJ, Huang Y, Horvath S, Leibovich BC, Chopra S, Liao SY, Stanbridge E, Lerman MI, Palotie A, Figlin RA, Belldegrun AS (2003) Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res 9(2):802–811

Carney WP (2007) Circulating oncoproteins HER2/neu, EGFR and CAIX (MN) as novel cancer biomarkers. Expert Rev Mol Diagn 7(3):309–319. doi:10.1586/14737159.7.3.309

Chia SK, Wykoff CC, Watson PH, Han C, Leek RD, Pastorek J, Gatter KC, Ratcliffe P, Harris AL (2001) Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma. J Clin Oncol 19(16):3660–3668

Choschzick M, Oosterwijk E, Muller V, Woelber L, Simon R, Moch H, Tennstedt P (2011) Overexpression of carbonic anhydrase IX (CAIX) is an independent unfavorable prognostic marker in endometrioid ovarian cancer. Virchows Arch 459(2):193–200. doi:10.1007/s00428-011-1105-y

Duffy MJ, Evoy D, McDermott EW (2010) CA 15–3: uses and limitation as a biomarker for breast cancer. Clin Chim Acta 411(23–24):1869–1874. doi:10.1016/j.cca.2010.08.039

Fang JS, Gillies RD, Gatenby RA (2008) Adaptation to hypoxia and acidosis in carcinogenesis and tumor progression. Semin Cancer Biol 18(5):330–337. doi:10.1016/j.semcancer.2008.03.011

Gatenby RA, Smallbone K, Maini PK, Rose F, Averill J, Nagle RB, Worrall L, Gillies RJ (2007) Cellular adaptations to hypoxia and acidosis during somatic evolution of breast cancer. Br J Cancer 97(5):646–653. doi:10.1038/sj.bjc.6603922

Hayes DF, Ethier S, Lippman ME (2006) New guidelines for reporting of tumor marker studies in breast cancer research and treatment: remark. Breast Cancer Res Treat 100(2):237–238. doi:10.1007/s10549-006-9253-5

Hussain SA, Ganesan R, Reynolds G, Gross L, Stevens A, Pastorek J, Murray PG, Perunovic B, Anwar MS, Billingham L, James ND, Spooner D, Poole CJ, Rea DW, Palmer DH (2007) Hypoxia-regulated carbonic anhydrase IX expression is associated with poor survival in patients with invasive breast cancer. Br J Cancer 96(1):104–109. doi:10.1038/sj.bjc.6603530

Ivanov S, Liao SY, Ivanova A, Danilkovitch-Miagkova A, Tarasova N, Weirich G, Merrill MJ, Proescholdt MA, Oldfield EH, Lee J, Zavada J, Waheed A, Sly W, Lerman MI, Stanbridge EJ (2001) Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. Am J Pathol 158(3):905–919. doi:10.1016/S0002-9440(10)64038-2

Kaluz S, Kaluzova M, Liao SY, Lerman M, Stanbridge EJ (2009) Transcriptional control of the tumor- and hypoxia-marker carbonic anhydrase 9: a one transcription factor (HIF-1) show? Biochim Biophys Acta 1795(2):162–172. doi:10.1016/j.bbcan.2009.01.001

Kock L, Mahner S, Choschzick M, Eulenburg C, Milde-Langosch K, Schwarz J, Jaenicke F, Muller V, Woelber L (2011) Serum carbonic anhydrase IX and its prognostic relevance in vulvar cancer. Int J Gynecol Cancer 21(1):141–148. doi:10.1097/IGC.0b013e318204c34f

Li G, Feng G, Gentil-Perret A, Genin C, Tostain J (2008) Serum carbonic anhydrase 9 level is associated with postoperative recurrence of conventional renal cell cancer. J Urol 180(2):510–513. doi:10.1016/j.juro.2008.04.024 discussion 513–514

Loncaster JA, Harris AL, Davidson SE, Logue JP, Hunter RD, Wycoff CC, Pastorek J, Ratcliffe PJ, Stratford IJ, West CM (2001) Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: correlations with tumor oxygen measurements and prognosis in locally advanced carcinoma of the cervix. Cancer Res 61(17):6394–6399

Lou Y, McDonald PC, Oloumi A, Chia S, Ostlund C, Ahmadi A, Kyle A, Auf dem Keller U, Leung S, Huntsman D, Clarke B, Sutherland BW, Waterhouse D, Bally M, Roskelley C, Overall CM, Minchinton A, Pacchiano F, Carta F, Scozzafava A, Touisni N, Winum JY, Supuran CT, Dedhar S (2011) Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. Cancer Res 71(9):3364–3376. doi:10.1158/0008-5472.CAN-10-4261

Mori I, Yang Q, Kakudo K (2002) Predictive and prognostic markers for invasive breast cancer. Pathol Int 52(3):186–194

Müller V, Riethdorf S, Rack B, Janni W, Fasching PA, Solomayer E, Aktas B, Kasimir-Bauer S, Zeitz J, Pantel K, Fehm T (2011) Prospective evaluation of serum tissue inhibitor of metalloproteinase 1 and carbonic anhydrase IX in correlation to circulating tumor cells in patients with metastatic breast cancer. Breast Cancer Res 13(4):R71. doi:10.1186/bcr2916

Potter C, Harris AL (2004) Hypoxia inducible carbonic anhydrase IX, marker of tumour hypoxia, survival pathway and therapy target. Cell Cycle 3(2):164–167

Potter CP, Harris AL (2003) Diagnostic, prognostic and therapeutic implications of carbonic anhydrases in cancer. Br J Cancer 89(1):2–7. doi:10.1038/sj.bjc.6600936

Robertson N, Potter C, Harris AL (2004) Role of carbonic anhydrase IX in human tumor cell growth, survival, and invasion. Cancer Res 64(17):6160–6165. doi:10.1158/0008-5472.CAN-03-2224

Schroder C, Witzel I, Muller V, Krenkel S, Wirtz RM, Janicke F, Schumacher U, Milde-Langosch K (2011) Prognostic value of intercellular adhesion molecule (ICAM)-1 expression in breast cancer. J Cancer Res Clin Oncol 137(8):1193–1201. doi:10.1007/s00432-011-0984-2

Siebels M, Rohrmann K, Oberneder R, Stahler M, Haseke N, Beck J, Hofmann R, Kindler M, Kloepfer P, Stief C (2011) A clinical phase I/II trial with the monoclonal antibody cG250 (RENCAREX(R)) and interferon-alpha-2a in metastatic renal cell carcinoma patients. World J Urol 29(1):121–126. doi:10.1007/s00345-010-0570-2

Stoner M, Saville B, Wormke M, Dean D, Burghardt R, Safe S (2002) Hypoxia induces proteasome-dependent degradation of estrogen receptor alpha in ZR-75 breast cancer cells. Mol Endocrinol 16(10):2231–2242

Tafreshi NK, Bui MM, Bishop K, Lloyd MC, Enkemann SA, Lopez AS, Abrahams D, Carter BW, Vagner J, Gobmyer SR, Gillies RJ, Morse DL (2012) Noninvasive detection of breast cancer

lymph node metastasis using carbonic anhydrases IX and XII targeted imaging probes. Clin Cancer Res 18(1):207–219. doi:10.1158/1078-0432.CCR-11-0238

Winum JY, Poulsen SA, Supuran CT (2009a) Therapeutic applications of glycosidic carbonic anhydrase inhibitors. Med Res Rev 29(3):419–435. doi:10.1002/med.20141

Winum JY, Scozzafava A, Montero JL, Supuran CT (2009b) Inhibition of carbonic anhydrase IX: a new strategy against cancer. Anti-Cancer Agents Med Chem 9(6):693–702

Woelber L, Kress K, Kersten JF, Choschzick M, Kilic E, Herwig U, Lindner C, Schwarz J, Jaenicke F, Mahner S, Milde-Langosch K, Mueller V, Ihnen M (2011) Carbonic anhydrase IX in tumor tissue and sera of patients with primary cervical cancer. BMC cancer 11:12. doi:10.1186/1471-2407-11-12

Woelber L, Mueller V, Eulenburg C, Schwarz J, Carney W, Jaenicke F, Milde-Langosch K, Mahner S (2010) Serum carbonic anhydrase IX during first-line therapy of ovarian cancer. Gynecol Oncol 117(2):183–188. doi:10.1016/j.ygyno.2009.11.029

Zavada J, Zavadova Z, Pastorekova S, Ciampor F, Pastorek J, Zelnik V (1993) Expression of MaTu-MN protein in human tumor cultures and in clinical specimens. Int J Cancer 54(2):268–274

Zavada J, Zavadova Z, Zat'ovicova M, Hyrsl L, Kawaciuk I (2003) Soluble form of carbonic anhydrase IX (CA IX) in the serum and urine of renal carcinoma patients. Br J Cancer 89(6):1067–1071. doi:10.1038/sj.bjc.6601264