

Quantification of Carbonic Anhydrase IX Expression in Tissue and Plasma of Renal Cell Carcinoma Patients

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Introduction

Carbonic anhydrase IX (CAIX) is a tumor-associated antigen expressed by many cancers including kidney cancer.¹ CAIX is believed to regulate cell proliferation in response to hypoxia and acid-base balance of the tumor microenvironment in favor of tumor progression.^{2,3} In renal cell carcinoma (RCC), the expression of CAIX is switched on by the loss of the tumor suppressor gene *VHL* and/or the hypoxic tumor microenvironment.^{1,4,5}

CAIX has been found as an important prognostic marker in clear cell RCC (CRCC): a high CAIX level in advanced stage CRCC correlates with longer survival and improved response to immunotherapy.^{1,2}

Previous studies of the prognostic significance of CAIX used immunohistochemistry, a subjective and semi-quantitative method, to measure CAIX expression. In this study, we established that CAIX levels can be measured using a more accurate and quantitative method, enzyme linked immunosorbent assay (ELISA), using patient serum and tumor tissue. Furthermore, we found that serum CAIX levels detected by ELISA correlate with the tumor burden in CRCC patients and could be used to differentiate CRCC from other types of kidney cancers.

Hypothesis

- CAIX levels quantified by ELISA in CRCC tumor tissue will correlate with the levels measured by the current standard procedure, immunohistochemical staining.
- CAIX levels measured by ELISA in pre-surgery serum may correlate with the CAIX levels in tumor tissue.
- Serum CAIX levels in CRCC patients may significantly differ from levels in non-CRCC patients; therefore CAIX serum levels may aid the differential diagnosis of RCC.
- Serum CAIX levels in CRCC patients may reflect tumor burden.

Material and Methods

Patients and Controls: This study included 40 RCC patients. Fresh frozen tissue samples were collected from 21 CRCC and 19 non-CRCC patients. Serum samples were collected from 18 CRCC and 14 non-CRCC patients.

Material and Methods (cont'd)

Tissue and Serum Preparation: The tumor diagnosis was confirmed with a frozen H&E section. 50-micron thick slices of each tumor were lysed in RIPA buffer with HALT Protease Inhibitors and centrifuged at 14000 rpm for 15 minutes. The supernatant was extracted and stored at -80°C. 500 µl serum samples were maintained at -80°C.

CAIX ELISA: The Quantikine Human CA9 Immunoassay (R&D Systems, Minneapolis, MN) was used to measure the CAIX levels of all tissue and serum samples. Microtiter plates were coated with capture antibody before samples and standards were added. Subsequently, detection antibody and substrate solution were added. The colorimetric density of all samples were measured by a microplate reader at 450 nm, and CAIX concentrations were calculated according to a standard curve.

CAIX Immunohistochemical Staining and Evaluation: Tissue sections of the 40 RCC cases were stained with an anti-CAIX antibody (Novus Biologicals, NB100-417, dilution 1:2000) using a biotin-free, multimer technology detection system (Ventana, AZ). The immunostain for CAIX expression was evaluated for the staining intensity as well as the percentage of stained cells. For CAIX staining intensity, strong circumferential membranous staining was scored a 3, weak circumferential membranous staining a 2, weak partial membranous staining a 1, and negative staining a 0 (Figure 1). The % of cells corresponding with each staining intensity was also recorded. The CAIX high-score was calculated by multiplying the highest CAIX intensity (0-3) by the % of cells exhibiting the highest CAIX intensity. The CAIX total-score was calculated by adding together the products of each of the CAIX intensities (0-3) and the % of cells exhibiting that respective staining intensity. For example, if 80% of the cells in a tumor had a score of 3 and 20% of the cells had a score of 2, then the CAIX-high score would be $80 \times 3 = 240$, and the CAIX-total score would be $(80 \times 3) + (20 \times 2) = 280$.

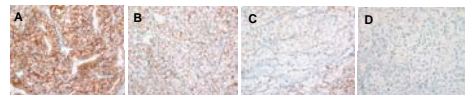


Figure 1: Immunohistochemical staining for CAIX. (A) Strong stain, score 3; (B) Weak stain, score 2; (C) Weak partial stain, score 1; (D) Negative stain, score 0.

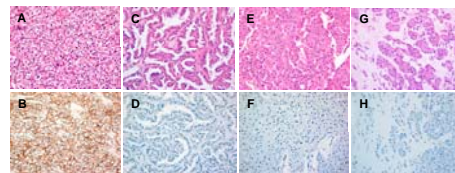


Figure 2: Immunohistochemical staining for CAIX in renal tumors. CAIX is strongly positive in CRCC (A & B), but negative in papillary RCC (C & D), chromophobe RCC (E & F), and oncocytoma (G & H).

Results

- CAIX levels in renal tumors detected by ELISA and immunohistochemistry are highly correlated with each other.

As shown in Table 1, the tissue CAIX levels detected by ELISA highly correlate with the CAIX levels detected by immunohistochemical stains that were represented by the % of cells positive for highest CAIX staining, the CAIX-high score, and % of cells positive for total CAIX staining, the CAIX-total score. The CAIX-total score has the highest correlation coefficient.

- CAIX levels in serum and tumor tissue detected by ELISA distinguish CRCC from non-CRCC.

Tumor tissues from CRCC had significantly higher CAIX levels assayed by ELISA than non-CRCC tissue (Table 2). In addition, serum CAIX levels from patients with CRCC are also significantly higher than those of patients bearing non-CRCC.

- Serum CAIX levels correlate with the tumor burden in patients with CRCC.

In patients with CRCC, the CAIX levels detected by ELISA significantly correlate with the tumor size and volume (Table 3).

- Serum CAIX levels do not correlate with tissue CAIX levels.

Table 1: CA9 levels in renal tumors: correlation between ELISA and immunohistochemistry

ELISA	Pearson correlation	Immunohistochemistry		
		% highest staining	CAIX-high score	CAIX-total score
	0.767	0.789	0.786	0.802
	P value	<0.001	<0.001	<0.001

Table 2: CAIX levels in serum and tumor tissue of patients with clear cell renal cell carcinoma and non-clear cell renal tumors

	Serum CAIX		Tissue CAIX	
	CRCC	Non-CRCC	CRCC	Non-CRCC
Case number	18	5	21	19
Mean (pg/ml)	126.1	2.5	77023.1	938.0
Range (pg/ml)	0-706.4	0-10.9	1272.7-192181.8	0-9000
P value (t test)	0.013		<0.001	

Table 3: Correlation between serum CAIX level and tumor size and volume of clear cell renal cell carcinoma

Serum CAIX	Pearson correlation	Tumor size	Tumor volume
		P value	P value
		0.498	0.535
		0.036	0.022

Conclusions

- CAIX levels in renal tumors detected by ELISA highly correlate with the standard immunohistochemical stain results. Therefore, ELISA is a sensitive and objective method to measure CAIX levels in renal tumors.
- CAIX serum and tissue levels in clear cell RCC detected by ELISA are significantly higher than those in non-clear cell RCC. Serum CAIX levels measured by ELISA may aid the differential diagnosis of RCC.
- Serum CAIX levels correlate with the tumor size and volume in patients with clear cell RCC.
- Serum CAIX levels do not correlate with tissue CAIX levels. Therefore, whether serum CAIX has prognostic significance needs further study.

Recommendations

- CAIX ELISA can be used for pre-surgical differential diagnosis of RCC.
- CAIX ELISA can be used in further studies in place of the current standard, immunohistochemical staining, in order to maximize the accuracy of CAIX as a CRCC prognostic marker.
- More studies need to be performed to ascertain the extent of the correlation of CAIX levels in serum with tumor size and volume vs. CAIX levels in tissue with tumor size and volume.

References

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