PLAGL1 (*ZAC1/LOT1*) Expression in Clear Cell Renal Cell Carcinoma: Correlations with Disease Progression and Unfavorable Prognosis

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Abstract. *Background: Pleiomorphic adenoma gene-like 1 (PLAGL1) protein was originally shown to induce cell-cycle arrest and promote apoptosis in several types of human tumors and therefore it was considered a candidate tumor suppressor. The involvement of PLAGL1 gene in the etiology and pathogenesis of clear cell renal cell carcinoma (ccRCC) has not been evaluated. The purpose of the present study was to determine the expression level of PLAGL1 in ccRCC and to investigate its potential utility as a prognostic factor. Materials and Methods: We applied quantitative real-time polymerase chain reaction (QPCR), western blotting and immunohistochemistry to measure PLAGL1 mRNA/protein contents in paired tumor and kidney specimens of 40 patients with ccRCC. Results: PLAGL1 mRNA and protein levels were increased in tumor tissues as determined by QPCR and immunohistochemistry, respectively. The average content of PLAGL1 protein measured by western blotting did not differ between tumor and non-cancerous kidney tissues. However, increased PLAGL1 protein level in ccRCC tissue positively correlated with the presence of distant metastases and worse patient outcome. Conclusion: These results suggest an oncogenic role of PLAGL1 in the progression of ccRCC and its potential value as a prognostic marker.*

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Clear cell renal cell carcinoma (ccRCC), the prevailing form of RCC, is characterized by the most aggressive behavior and poor prognosis among all types of kidney cancer (1-3). Although ccRCC tumors can be removed surgically, haematogeneous metastases frequently occur in the early stage of the disease and 35-65% of patients develop metastatic disease after nephrectomy (3). Over the past decade, the background of ccRCC pathogenesis has been extensively screened for molecular biomarkers and relevant gene signatures, however, only few have significant prognostic value which can be used in clinical practice (3-5).

Pleiomorphic adenoma gene-like 1 (*PLAGL1*, also known as *ZAC1* or *LOT1*) gene belongs to the group of imprinted genes and encodes a zinc finger transcription factor. *PLAGL1* is expressed in numerous types of embryonic and adult human tissues (6, 7). PLAGL1 protein plays a critical role in the integration and synergizing of multiple regulatory pathways during development and postnatal life (8). PLAGL1 acts as a transcription factor by sequence-specific DNA binding in promoter regions of target genes (6). It can also bind to other proteins, such as nuclear receptors, p53 and histone acetyltransferases, and modulate their transcriptional or enzymatic capacity (7, 9, 10).

PLAGL1 is considered a tumor suppressor because it shares its activity with p53, inducing cell-cycle arrest and apoptosis of cancer cells (10). Reduced or loss of *PLAGL1* expression correlates with incidence and progression of several human neoplasms, including breast and ovary cancer, pituitary tumors and basal cell carcinoma (6, 7). Recently, we demonstrated that *PLAGL1* is down-regulated in tumor tissue and cell lines of colorectal cancer (CRC). We found decreased PLAGL1 protein content that correlated with involvement of lymph nodes, incidence of distant metastasis and progression of TNM stage in CRC (11).

The possible role of *PLAGL1* in the etiology and pathogenesis of RCC has not been elucidated. Increased *PLAGL1* cDNA content was detected in the tumor tissue of one patient with RCC but there were no further findings (12).

In the present study, we investigated *PLAGL1* gene expression in ccRCC tumor tissue in comparison to unchanged kidney. The association of PLAGL1 protein levels with the incidence of distant metastasis in patients with ccRCC patients and their outcome was assessed.

Materials and Methods

Patients and collection of tissue samples. This study was carried out in accordance with the principles and procedures of the Bioethics Committee for Scientific Research at the University of Warmia and Mazury in Olsztyn, Poland (agreements no. 4/2010 and 33/2010). Written informed consent regarding the use of tissue was obtained from each patient included in the study. Specimens were obtained from postoperative material of 40 patients with ccRCC who were operated on at the Department of Oncological Surgery, Warmia and Mazury Oncological Center in Olsztyn in the period between March 2010 and August 2012. All specimens qualified for the study were verified by a pathologist. None of the patients had suffered from a second neoplastic disease or other serious disease. The clinical characteristics and their survival data of the patients were collected during the study.

Paired tissue fragments were obtained immediately after nephrectomy from ccRCC primary tumor tissue and unchanged kidney tissue of the same patient. Tumor and kidney tissue samples for RNA or protein extraction were snap-frozen in liquid nitrogen and stored at −80˚C until further analyses. Tumor and kidney fragments for histological and immunohistochemical studies were placed in 4% buffered formaldehyde and processed into paraffin blocks. The tumor stage was characterized by pathologist according to the TNM system (American Joint Committee on Cancer) (13). Hematoxylin and eosin (H&E)-stained sections of collected tumor and matching kidney tissues were evaluated to confirm their cancer or cancer-free phenotype, respectively. The degree of tumor malignancy was determined using the G scale of the Fuhrman nuclear grading system (14).

RNA extraction, reverse transcription and quantitative real-time polymerase chain reaction (QPCR). Total RNA was isolated, reverse transcribed and quantified for PLAGL1 expression according to the modified method described by Kowalczyk *et al*. (11). Briefly, the level of *PLAGL1* mRNA in tissue homogenates was determined by QPCR and normalized to that of TATA box binding protein (*TBP*) mRNA content using the respective TaqMan® Gene Expression Assay (*PLAGL1*: Hs00414677_m1; *TBP*: Hs00427620_m1; Applied Biosystems, Foster City, CA, USA). The ΔΔCt method was used to determine the fold differences in expression between the paired samples of ccRCC tumor and unchanged kidney. Based on the median *PLAGL1* transcript content in ccRCC tumor tissue the specimens were divided into two groups regarded as having a 'low' or 'high' *PLAGL1* mRNA expression level.

Protein extraction, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. Protein extraction, SDS-PAGE and immunoblotting of PLAGL1 protein were carried out as described previously (11). Briefly, the level of PLAGL1 protein in tissue homogenates was measured using rabbit anti-human antibodies against PLAGL1 (dilluted 1:1000, #ab129063; Abcam, Cambridge, UK) and actin (ACTB) (dilluted 1:100, #A2066; SigmaAldrich, St. Louis, MO, USA) as the internal protein load control. PLAGL1/ACTB OD ratios were used to determine fold differences in expression between ccRCC and corresponding kidney tissues. Based on the median PLAGL1 protein content in ccRCC tumor tissue, the specimens were divided into two groups regarded as having a 'low' or 'high' PLAGL1/ACTB OD ratio.

Immunohistochemical (IHC) staining and evaluation of immunoreactivity. Paraffin sections of ccRCC and unchanged kidney tissues were subjected to IHC according to the protocol described elsewhere (15). Briefly, rabbit anti-human antibody against PLAGL1 (diluted 1:2000 in phosphate-buffered saline, #ab129063; Abcam) was used. The immunoreactivity of PLAGL1 was evaluated by pathologist who was blinded to the clinicopathological data of the patients. A five-grade scoring scale, based on the intensity of reaction in ccRCC tumor sections or epithelial cells of proximal convoluted tubules (PCTs) in sections of unchanged kidney was used: 0: negative, 1: trace, 2: weak, 3: moderate and 4: strong staining intensity. To test the correlations between the clinicopathological data and PLAGL1 staining intensity in ccRCC tumor tissue, the specimens were divided into two groups regarded as 'weak' (IHC score below the median value, *i.e.* 0-2) and 'strong' PLAGL1 staining intensity (IHC score equal and above the median value, *i.e.* 3-4).

Statistical analyses were performed by Prism (v. 6.04; Graphpad, La Jolla, CA, USA) and Statistica (v.10; Statsoft, Tulsa, OK, USA) programs. The differences in *PLAGL1* mRNA and protein levels in paired tumor and unchanged kidney samples were examined by Wilcoxon matched-pairs test. The relationships between the clinicopathological data and the *PLAGL1* mRNA or protein levels in the ccRCC tissue were analyzed by Fisher's exact test and confirmed by Mann–Whitney *U*-test. Patients' overall survival fractions were calculated and visualized according to the Kaplan–Meier method. To investigate the differences between overall survival in different patient groups, the log-rank test and Cox proportional hazard regression were used. For all performed analyses, the results were considered statistically significant for $p<0.05$.

Results

PLAGL1 mRNA is elevated in ccRCC. Forty paired tissue homogenates of ccRCC tumor and unchanged part of kidney were subjected to QPCR analysis. All tested tumor and non-cancerous tissue samples exhibited the presence of *PLAGL1* mRNA. The average expression of *PLAGL1* mRNA was significantly increased in the tumor tissues in comparison to unchanged kidney tissue (relative expression: 1.67±0.20 *vs*. 1.00±0.10, $p=0.0002$; Figure 1). No relationships between *PLAGL1* mRNA and clinicopathological parameters were found (Table I).

PLAGL1 protein level correlates positively with the incidence of distant metastasis in ccRCC. To determine PLAGL1 protein levels, 40 paired specimens were subjected to western blotting. PLAGL1 protein was found in all analyzed samples. The mean

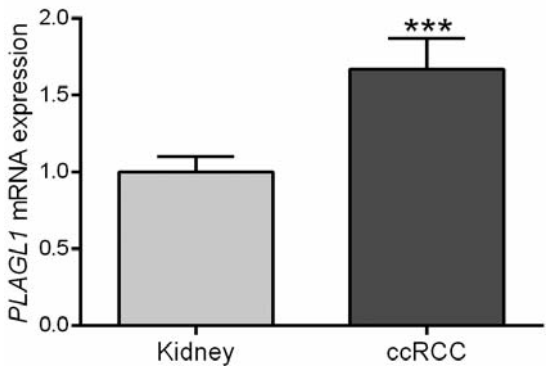

Figure 1. *Pleiomorphic adenoma gene-like 1 (PLAGL1) mRNA levels in clear cell renal cell carcinoma (ccRCC) tumor in relation to the corresponding unchanged kidney tissue determined by quantitative real-time polymerase chain reaction. Data are presented as the mean±SEM (N=40); ***p<0.001.*

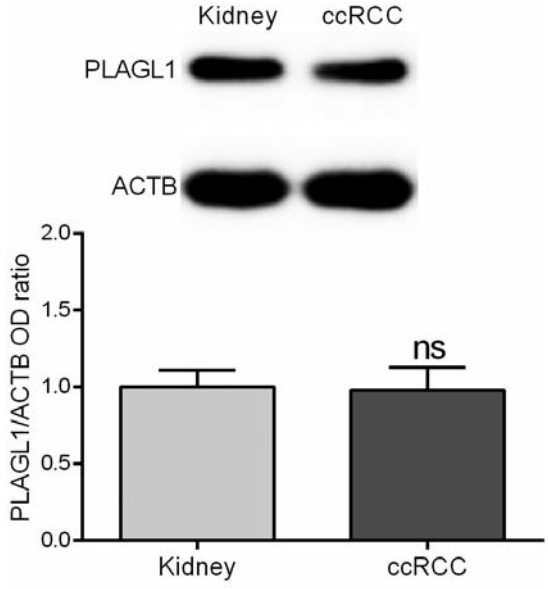

Figure 2. *Pleiomorphic adenoma gene-like 1 (PLAGL1) protein levels in clear cell renal cell carcinoma (ccRCC) tumor and corresponding unchanged kidney tissue determined by western blotting. Representative blots of studied tissues are shown. Actin (ACTB) was used as a loading control. Data are presented as the mean±SEM (N=40); ns: not significant, p>0.05.*

levels of PLAGL1 protein in paired tumor and noncancerous kidney specimens were similar and did not differ significantly (0.98±0.15 *vs*. 1.00±0.11, respectively; p=0.4030; Figure 2). However, the expression levels of PLAGL1 protein in tumor tissue positively correlated with the presence of distant metastases (p=0.0471; Table II) and we recorded an almost 3-fold higher average content of PLAGL1 in the tumors of metastasized ccRCC cases when compared to M0 patients (2.79±0.74 *vs*. 1.06±0.17, respectively; p=0.0105; Figure 3).

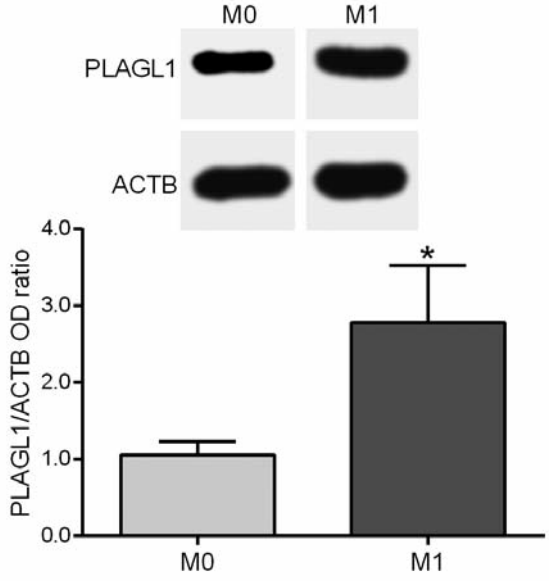

Figure 3. *Pleiomorphic adenoma gene-like 1 (PLAGL1) protein levels in primary clear cell renal cell carcinoma (ccRCC) tumor tissue of patients with non-metastatic (M0; N=35) and metastatic (M1; N=5) ccRCC determined by western blotting. Representative blots of studied tissues are shown. Actin (ACTB) was used as a loading control. Data are presented as the mean±SEM; *p<0.05.*

Clear cell RCC tumor cells showed strong PLAGL1 immunoreactivity. IHC staining of 37 paired sections of ccRCC tumor and non-cancerous kidney tissue demonstrated the presence of PLAGL1 in the cancer cells of 36/37 (97.3%) ccRCC cases and in the epithelium of PCTs of 34/37 (91.9%) unchanged kidney samples. PLAGL1 immunoreactivity was localized in the cytoplasm and, to a lesser extent, in the nuclei of both PCT and ccRCC tumor cells (Figure 4A and 4B, respectively). The average intensity of PLAGL1 staining was stronger in ccRCC tumor cells than in PCT epithelium (2.89±0.15 *vs*. 1.79±0.18, p<0.0001; Figure 5). We did not find any correlations between PLAGL1 immunoreactivity in the tumor tissue and clinicopathological parameters of patients with ccRCC (Table III).

Higher PLAGL1 protein levels in ccRCC tissue homogenates correlate with unfavorable prognosis. To determine the possible utility of *PLAGL1* expression at the mRNA and protein levels as prognostic markers, patients with ccRCC were followed-up and the median observation period was 37.1 months. During this time 15/40 (37.5%) patients died.

PLAGL1 protein level in the tumor tissue was significantly associated with shorter overall survival of patients with ccRCC (p=0.0319; Figure 6B). The hazard ratio (HR) of death was three-fold higher in the group of patients with high expression of PLAGL1 protein in the

Table I. *Correlations between clinicopathological characteristics of patients with clear cell renal cell carcinoma (ccRCC) and pleiomorphic adenoma gene-like 1 (PLAGL1) mRNA expression (by quantitative real-time polymerase chain reaction) in homogenates of tumor tissue.*

Number of cases	*PLAGL1* mRNA level				*p*-Value
	Low		High		
	n	(%)	n	(%)	
All patients	40	20	50.0%	20	50.0%	
Men	21	12	57.1%	9	42.9%	0.5273[a]
Women	19	8	42.1%	11	57.9%	
Primary tumor status						
T1	21	12	57.1%	9	42.9%	0.5273[a]
T2+T3	19	8	42.1%	11	57.9%	
Malignancy grade						
G1+G2	33	18	54.5%	15	45.5%	0.4075[a]
G3+G4	7	2	28.6%	5	71.4%	
Metastasis						
M0	35	16	45.7%	19	54.3%	0.3416[a]
M1	5	4	80.0%	1	20.0%	
Age (mean±SD), years		63.3±11.3		60.1±12.4		0.2327[b]
Primary tumor size (mean±SD), cm		6.5±2.8		8.1±3.3		0.1627[b]

[a]Fisher's exact test; [b]Mann-Whitney *U*-test.

Table II. *Correlations between clinicopathological characteristics of studied patients with clear cell renal cell carcinoma (ccRCC) and pleiomorphic adenoma gene-like 1 (PLAGL1) protein levels (western blotting) in homogenates of tumor tissue.*

Number of cases	PLAGL1 protein level				*p*-Value
	Low		High		
	n	(%)	n	(%)	
All patients	40	20	50.0%	20	50.0%	
Men	21	8	38.1%	13	61.9%	0.2049[a]
Women	19	12	63.2%	7	36.8%	
Primary tumor status						
T1	21	13	61.9%	8	38.1%	0.2049[a]
T2+T3	19	7	36.8%	12	63.2%	
Malignancy grade						
G1+G2	33	16	48.5%	17	51.5%	1.0000[a]
G3+G4	7	4	57.1%	3	42.9%	
Metastasis						
M0	35	20	57.1%	15	42.9%	**0.0471**[a]
M1	5	0	0.0%	5	100.0%	
Age (mean±SD), years		60.3±14.6		63.6±7.7		0.6430[b]
Primary tumor size (mean±SD), cm		7.1±3.8		7.5±2.4		0.4647[b]

[a]Fisher's exact test; [b]Mann-Whitney *U*-test; Significant *p*-values (<0.05) are given in bold.

tumor tissue when compared to those with low PLAGL1 protein content (Table IV). However, the multivariate analysis did not confirm that the PLAGL1 protein expression can be considered as an independent marker of a worse patient outcome (Table IV).

Primary tumor status, the degree of tumor malignancy and the presence of distant metastases significantly affected survival rates. Other investigated variables, including *PLAGL1* expression at the mRNA level and PLAGL1 IHC score, did not correspond with patient outcome, even when high HRs were calculated (Figure 6A and C; Table IV).

Discussion

Clear cell RCC is the most frequent and malignant kidney neoplasm arising from epithelial cells of the PCTs (16). Despite significant progress in the understanding of the molecular mechanisms underlying ccRCC biology, the best established prognostic factors are still based on tumor morphology and clinical progression (3-5). Searching for genes that are differentially expressed in tumor and non-cancerous tissue, investigation of their association with clinicopathological parameters and further validation in clinical practice can allow identification of new diagnostic, prognostic and predictive molecular biomarkers (3-5).

PLAGL1 was mapped to chromosome 6 at q24-q25, a region that is frequently rearranged in cancer (6, 17). It was reported that 4/22 (18%) non-papillary RCCs exhibited loss of heterozygosity in chromosome 6q (18) and this cytogenetic change always occurred in combination with allelic loss at chromosome 3p, the locus of the von Hippel-Lindau (*VHL*) gene. Since it has been suggested that chromosome 6q may harbor at least one potent tumor-suppressor gene, and rearrangements of 6q region can contribute to the etiology of ccRCC (18), *PLAGL1* gene appeared to be a promising candidate (6, 17). Interestingly, a preliminary observation of a 3-fold higher *PLAGL1* cDNA level in RCC tissue than in noncancerous region of kidney of one patient (12) was not confirmed by the same group using mRNA isolated from RCC tissue from patients and kidney cancer cell line, 786-O (12, 19). Therefore, we decided to assess the expression of *PLAGL1* at both mRNA and protein levels in tumor and matched noncancerous kidney tissues of patients with ccRCC. The relationships between the molecular data and patients' clinicopathological parameters, including overall survival, were also investigated.

In the present study, we demonstrated an increased expression of *PLAGL1* in ccRCC tissue compared to non-cancerous kidney tissue. The average levels of *PLAGL1* mRNA and PLAGL1 immunostaining were significantly elevated in the cancerous tissue; however, the PLAGL1 protein content measured by western blotting was similar in tissue homogenates of tumor and noncancerous kidney tissues. This

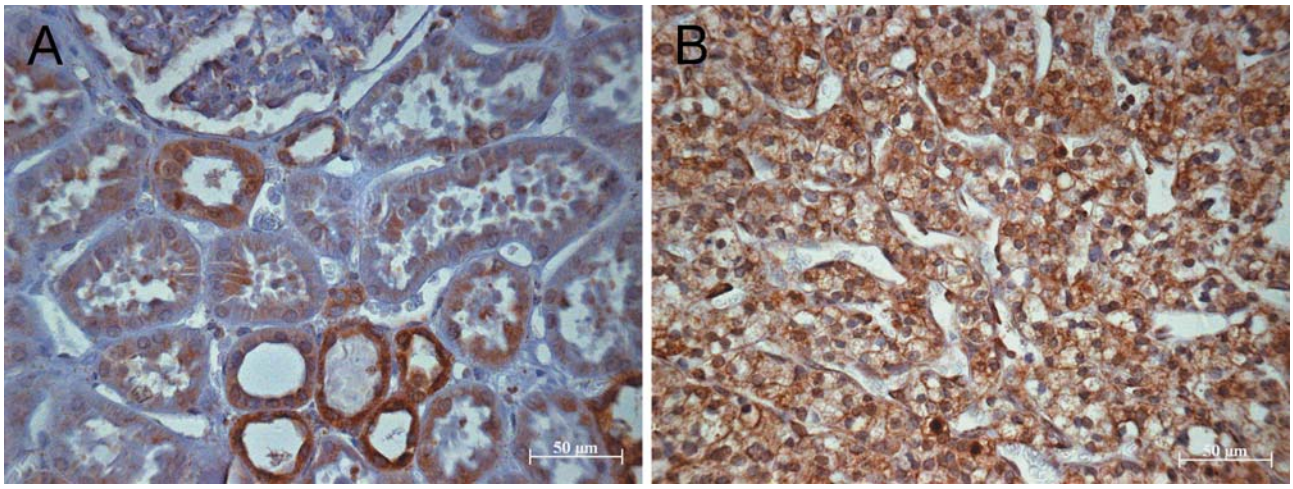

Figure 4. *The evaluation of pleiomorphic adenoma gene-like 1 (PLAGL1) protein expression in sections of unchanged kidney (A) and clear cell renal cell carcinoma (ccRCC) tumor tissue (B) by immunohistochemistry. Representative immunostainings are shown. PLAGL1 expression was weak to moderate in the epithelial cells of proximal tubules and strong in distal tubules and collecting ducts (A), while cancer cells revealed relatively strong PLAGL1 immunoreactivity (B). PLAGL1 immunoreactivity was localized mainly in the cytoplasm, but it was also present in the nuclei of epithelium of proximal tubules and ccRCC tumor cells. Original magnification, ×200.*

Table III. *Correlations between clinicopathological characteristics of the studied patients with clear cell renal cell carcinoma (ccRCC) and pleiomorphic adenoma gene-like 1 (PLAGL1) staining intensity in tumor cells.*

	Number of cases	PLAGL1 IHC intensity				*p*-Value
		Weak (score 0-2)		Strong (score 3-4)		
		n	(%)	n	(%)	
All patients	37	9	24.3%	28	75.7%	
Men	20	4	20.0%	16	80.0%	0.7034[a]
Women	17	5	29.4%	12	70.6%	
Primary tumor status						
T1	19	5	26.3%	14	73.7%	1.0000[a]
T2+T3	18	4	22.2%	14	77.8%	
Malignancy grade						
G1+G2	30	7	23.3%	23	76.7%	1.0000[a]
G3+G4	7	2	28.6%	5	71.4%	
Metastasis						
M0	32	7	21.9%	25	78.1%	0.5773[a]
M1	5	2	40.0%	3	60.0%	
Age (mean±SD), years			60.3±12.5		61.61±12.0	0.8546[b]
Primary tumor size (mean±SD), cm			7.5±3.7		7.5±2.9	0.7737[b]

[a]Fisher's exact test; [b]Mann–Whitney *U*-test. IHC: Immunohistochemical staining.

discrepancy can be explained by our recent finding of the prevalence of PLAGL1 immunoreactivity in distal parts of nephron and collecting ducts in human kidney (15). This implies that majority of the PLAGL1 protein in the homogenates of unchanged kidney did not derive from proximal tubules, but from distal parts of the uriniferous tubules. The latter can explain our finding of similar PLAGL1 protein levels in ccRCC, which develops from the epithelium of proximal tubules, and noncancerous kidney tissue.

Although the results of our study are in line with the only available study performed on kidney tumor that revealed elevated PLAGL1 expression in RCC tissue (12), they are in

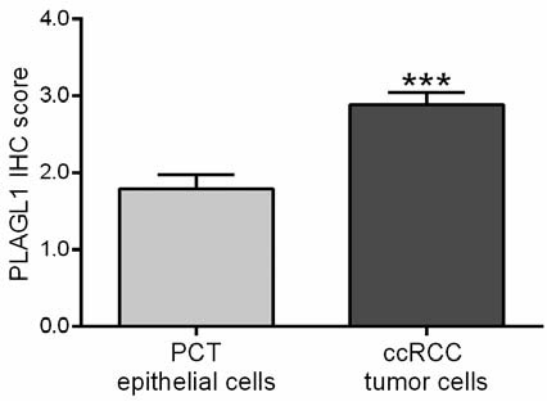

Figure 5. *The intensity of pleiomorphic adenoma gene-like 1 (PLAGL1) immunostaining in clear cell renal cell carcinoma (ccRCC) tumor cells and proximal tubules (PCT) of unchanged kidney, as determined by immunohistochemistry. Data are presented as the mean±SEM (N=37); ***p<0.001.*

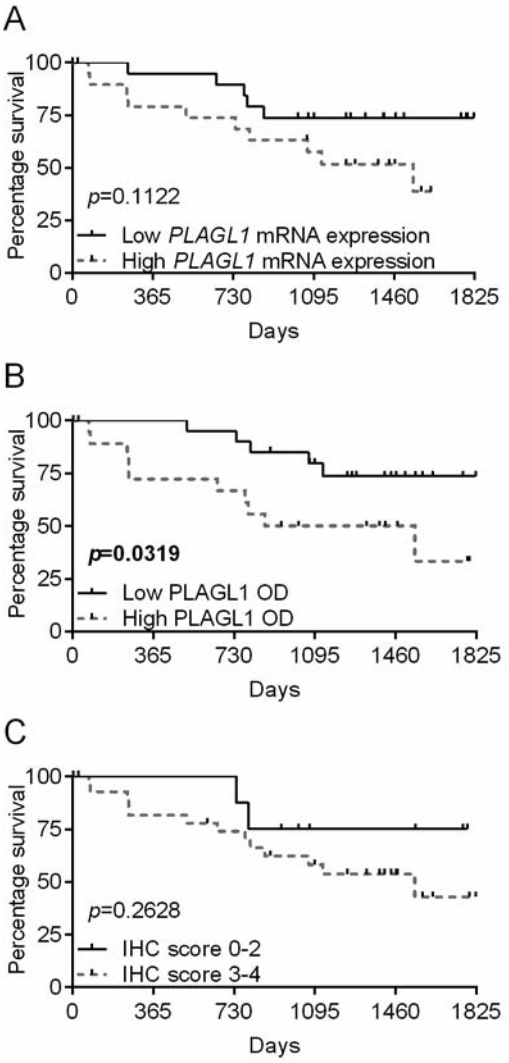

Figure 6. *Kaplan–Meier diagrams of survival for patients with clear cell renal cell carcinoma (ccRCC) and corresponding log-rank test. p-Values are shown related to pleiomorphic adenoma gene-like 1 (PLAGL1) expression at the mRNA (A) and protein (B and C) levels. The significant p-value (<0.05) is given in bold. IHC: Immunohistochemical staining.*

opposition to the general concept of antiproliferative and proapoptotic role of PLAGL1 proposed by other investigators. The majority of studies that explored the role of PLAGL1 in different types of neoplasms and cancer cell lines reported loss or reduced expression of *PLAGL1*, emphasizing on its tumor-suppressor properties and close associations with the p53-dependent pathways (6, 7, 10, 17). Interestingly, the studies performed on mice showed that knockout of expressed *Plagl1* allele (*i.e.* paternal) results in intrauterine growth restriction, impaired bone formation and reduced survival of pups, proving that PLAGL1 is indispensable for a normal growth and cell differentiation (8). There exist only limited data showing the overexpression of PLAGL1 in tumors and cancer cell lines. It was recently reported that increased *PLAGL1* mRNA correlated with shorter periods of metastasis-free and overall survival in patients with undifferentiated sarcomas (20). The overexpression of *Plagl1* in the murine glioma cell line, NSCL61, was shown to induce a tumorigenic phenotype of these cells (21). The treatment of human colorectal (HTB-38 and CCL-247) and breast (HTB-22) cancer cell lines with carcinogens resulted in up-regulation of *PLAGL1* transcription (22). *PLAGL1* may also be overexpressed in embryonal cancer and cancer cell lines derived from these tumors. Silencing of *PLAGL1* in a rhabdomyosarcoma cell line showed that PLAGL1 promoted proliferation and growth (23). Moreover, cells exposed to *PLAGL1* siRNA revealed reduced expression levels of other imprinted genes, including tumorigenic insulin-like growth factor 2 (*IGF2*) (24). It was suggested that epigenetic mechanisms, in particular DNA demethylation followed by loss of imprinting (LOI) can be responsible for *PLAGL1* and *IGF2* overexpression in the analyzed cell lines (22, 24). Interestingly, LOI can also contribute to the development and progression of common adult cancer, including RCC, *e.g.* de-methylation and bi-allelic expression of *IGF2* was revealed in 56% and 50% of analyzed RCC cases, respectively (25, 26). LOI of *IGF2* resulted in the up-regulation of its expression and correlated with RCC tumor progression. Since both *IGF2* and *PLAGL1* are often expressed in a coordinated manner (8, 23), the mechanisms underlying *PLAGL1* overexpression in ccRCC might also comprise epigenetic alternations, but this has to be elucidated in further studies.

Our finding of higher PLAGL1 protein level in the primary tumor tissue of patients with metastatic ccRCC than in the samples of those with non-metastatic disease suggests

Table IV. *Univariate and Multivariate Cox proportional hazard regression of overall survival for patients with clear cell renal cell carcinoma.*

Parameter	Deaths	Cases	(%)	Univariate Cox regression			Multivariate Cox regression		
				HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
All patients	15	40	37.5%						
PLAGL1 mRNA level									
Low	5	20	25.0%	(1.00)					
High	10	20	50.0%	2.32	0.79-6.81	0.1238			
PLAGL1 protein level									
Low	5	20	25.0%	(1.00)			(1.00)		
High	10	20	50.0%	3.06	1.04-9.00	**0.0418**	2.73	0.67-11.11	0.1601
PLAGL1 IHC intensity									
Weak	2	9	22.2%	(1.00)					
Strong	13	28	46.4%	2.29	0.51-10.18	0.2772			
Gender									
Men	8	21	38.1%	(1.00)					
Women	7	19	36.8%	0.93	0.34-2.58	0.8911			
Primary tumor status									
T1	2	21	9.5%	(1.00)			(1.00)		
T2+T3	13	19	68.4%	8.15	1.83-36.36	**0.0060**	4.59	0.89-23.71	0.0689
Malignancy grade									
G1+G2	9	33	27.3%	(1.00)			(1.00)		
G3+G4	6	7	85.7%	3.69	1.30-10.49	**0.0142**	2.48	0.63-9.73	0.1925
Metastasis									
M0	11	35	31.4%	(1.00)			(1.00)		
M1	4	5	80.0%	3.58	1.13-11.33	**0.0304**	0.78	0.17-3.55	0.7532
Age	15	40	37.5%	1.00	0.95-1.04	0.8460			
Primary tumor size	15	40	37.5%	1.11	1.01-1.28	0.1618			

HR: Hazard ratio, CI: confidence interval. Significant *p*-values (<0.05) are given in bold. IHC: Immunohistochemical staining.

that PLAGL1 could serve as a possible protein marker of ccRCC progression. As metastasis is a major cause of death from ccRCC, the estimation of recurrence risk is a key issue during surveillance after nephrectomy. Currently, there are several reliable molecular markers for tumor tissue and peripheral blood that are used in predicting ccRCC tumor progression. They include carbonic anhydrase (CA9), vascular endothelial growth factor and p53, which are useful in the prediction of metastasis, while other factors are used to assess responsiveness to certain pharmacotherapies (3, 4). Since we observed the association of increased PLAGL1 level with patient outcome, a longer observation period is required to evaluate and validate prognostic value of PLAGL1 in the progression of ccRCC. Further studies should also aim to identify whether PLAGL1 could be combined with already validated biomarkers to create a gene/protein signature useful in the prediction of ccRCC progression and patient prognosis.

In conclusion, we present the first comprehensive investigation providing evidence for the up-regulation of *PLAGL1* gene in ccRCC and suggesting its oncogenic role in the progression of this cancer type. The measurement of PLAGL1 protein in ccRCC can serve as a potential marker for increased risk of distant metastasis and shorter patient survival, although further studies are needed to validate its prognostic significance.

Acknowledgements

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