Prognostic impact of phosphatidylinositol phosphate and its associated enzyme levels in patients with renal cell carcinoma treated surgically



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Introduction

- ➤ The involvement of lipid metabolism in development and progression of renal cell carcinoma (RCC) has been reported. However, the role of phospholipid profile alterations in RCC has not yet been systematically evaluated.
- Phosphatidylinositol phosphates (PIPs), which are important regulatory membrane lipids, are involved in many cellular processes including cancer progression. Sasaki, Progress in Lipid Research, 2009
- ➤ PIPs pathway is associated with risk of RCC, and PI3K/AKT, phosphatidylinositol 3-kinase pathway has been known to be highly activated in RCC.
- Tan J, Sci Rep, 2015, Guo, J Genet Geomics, 2016

 ➤ Here, we investigated PIPs profile including their phosphorylated forms (PI, PIP₁ and PIP₂) in human RCC tissues with an original method using mass spectrometry.

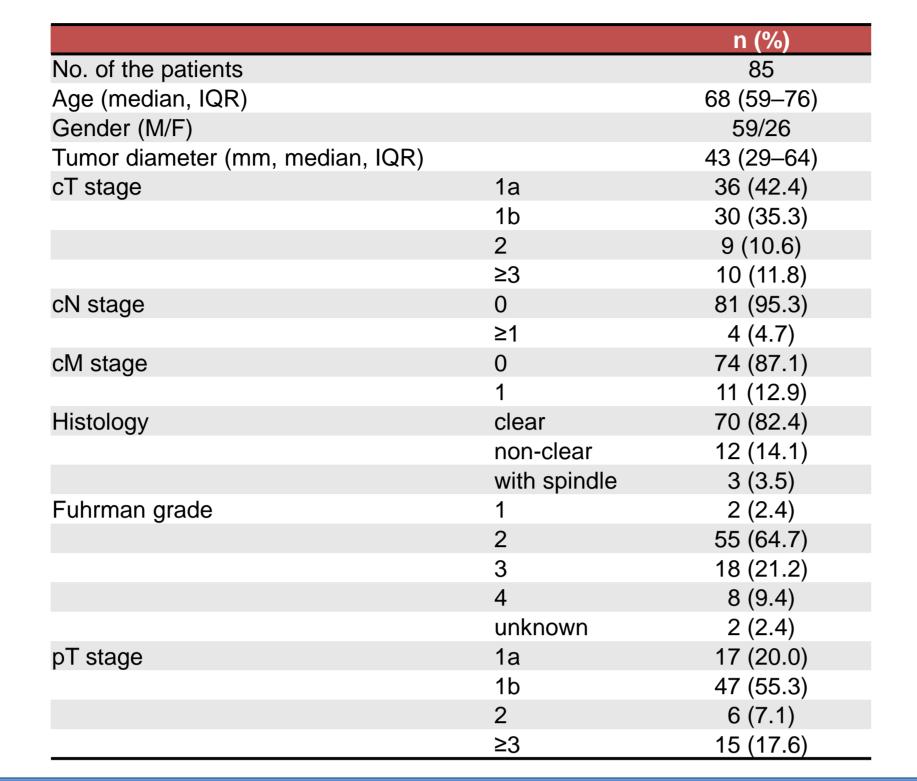
Materials and methods

- ➤ A total of 85 RCC patients treated with surgery at Akita University Hospital between 2011 to 2014 were included in the analyses. Table. 1
- ➤ The level of PIPs including PI, PIP₁ and PIP₂ in the tumor and its surrounding normal renal tissue was measured using mass spectrometry.
- Sample preparation for mass spectrometry was performed according to the previous reports (Clark et al. Nature Methods, 2011). 17:0/20:4-phosphatidylinositol 4-monophosphate, 17:0/20:4-phosphatidylinositol 4,5-bisphosphate and 17:0/20:4-phosphatidylinositol 3,4,5-trisphosphate (Avanti Polar Lipids, Inc. Alabaster, AL) were added to lysates as internal/surrogate standards.
- LC-MS/MS analysis was performed with an Ultimate 3000 LC system (Thermo Fisher Scientific) connected in tandem to a TSQ Vantage triple stage quadrupole mass spectrometer (Thermo Fisher Scientific). Selected reaction monitoring (SRM) were employed for quantitation of phosphoinositide species using a pre-set list of the mass to charge ratio (m/z) values. The parent ions were selected in the first quadrupole, subjected to fragmentation with collision-induced dissociation, and the product ions were monitored. Peak areas of individual species were normalized to the internal/surrogate standards having a heptadecanoyl moiety.
- The mRNA expression of 16 phosphatases associated with PIPs pathways in the tumor and its surrounding normal renal tissue was measured by qRT- PCR. Table 2
- Principal component analysis (PCA) was performed by MetaboAnayist software.

Table 2. Candidate phosphoinositide phosphatases evaluated in the current study

PTEN	MTMR7
TPTE2	MTMR14
MTM1	INPP4A
MTMR1	TMEM55A
MTMR2	TMEM55B
MTMR3	OCRL
MTMR4	SYNJ1
MTMR6	SYNJ2

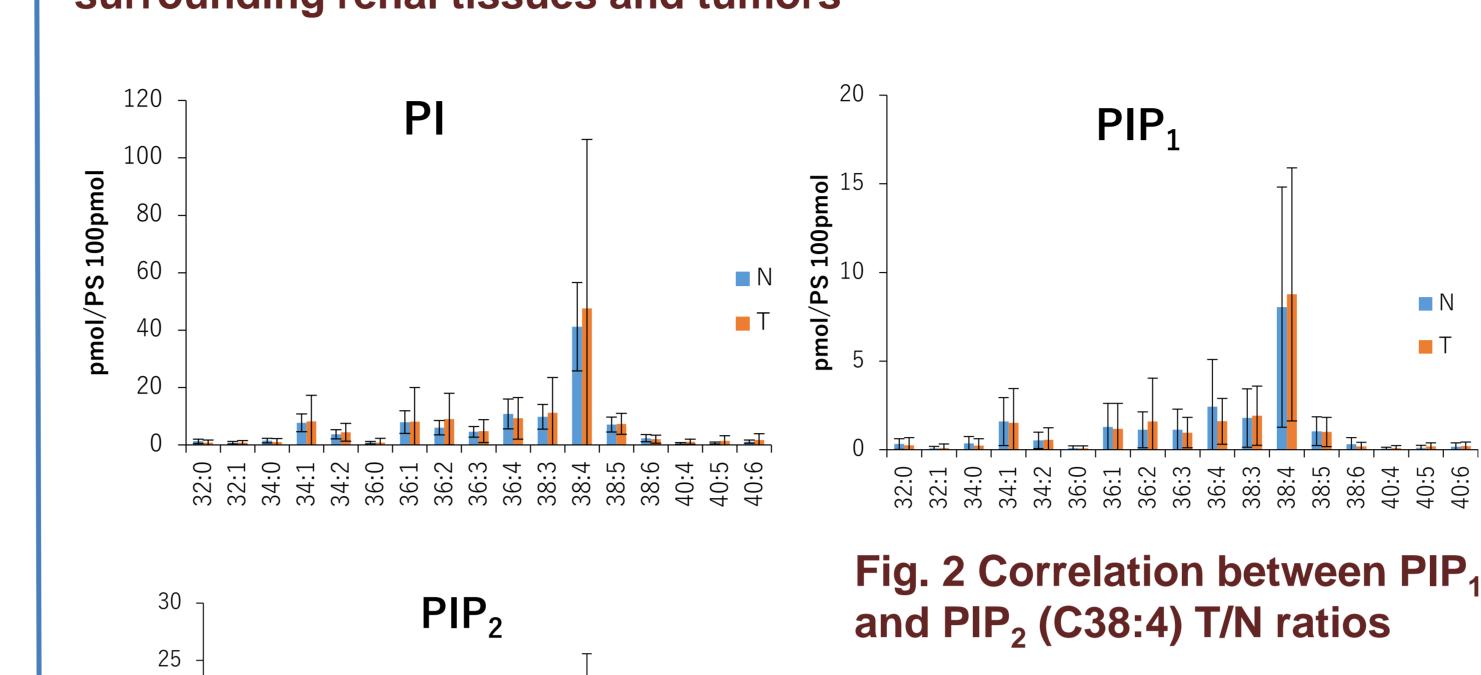
Table 1. Patient characteristics

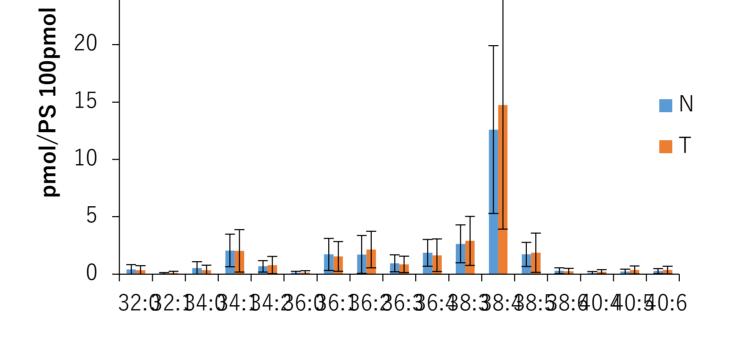


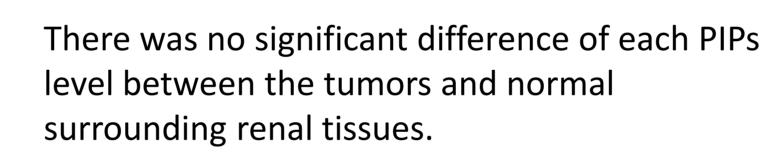
Results

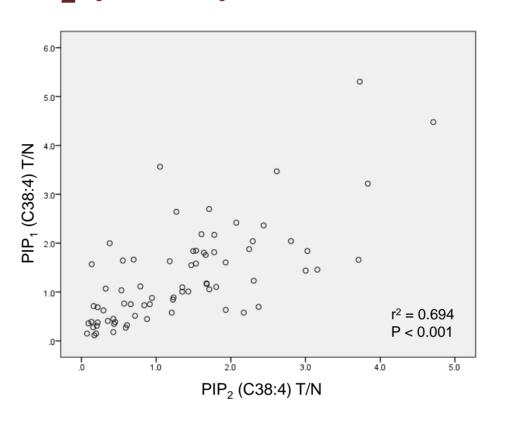
- ➤ There were no significant difference of each PIPs level between the tumors and normal surrounding tissues Fig.1
- ➤ The PIP₁ tumor to normal (T/N) ratio was strongly correlated with the PIP₂ T/N ratio. Fig. 2
- ➤ The PIP₁ and PIP₂ profiles can be dichotomized into two groups based on the difference of the levels between tumor and normal surrounding renal tissue. Fig. 3
- ➤ The patients with high grade tumor had significantly lower PIP₁ and PIP₂ (C38:4) T/N ratio. Fig.4
- The patients with high stage tumor tended to have lower PIP₂ (C38:4) T/N ratio. Fig 5
- Metastatic-free survival in patients with low PIP₁ and PIP₂ (C38:4) T/N ratio was significantly shorter than that in patients with high PIP₁ and/or PIP₂ (C38:4) T/N ratio (p = 0.007), whereas there was no difference of cancer-specific survival the two groups (p = 0.590). Fig.6
- ➤ The 5-year metastatic-free survival (MFS) in patients with low PIP1 and PIP2 (C38:4) T/N ratio was 67.0%, whereas that in patients having at least one of high PIP₁ or PIP₂ T/N ratio was 96.2%. Fig. 6
- ➤ In multivariable analysis, a pT stage and a low PIP1 and PIP2 T/N ratio were independent prognostic factor for poor MFS in patients with RCC who underwent surgery (HR = 6.8, p = 0.015, HR = 9.23, p = 0.041, respectively). Table 3
- Several candidate phosphatases associated with high grade tumor were detected by VIP score. Fig. 7
- Among those phosphatases, the T/N ratios of MTM1 and MTMR6 mRNA were highly correlated with PIP₁ and PIP₂ (C38:4) T/N ratio. Table 4

Fig. 1. The difference of PIPs levels between normal surrounding renal tissues and tumors



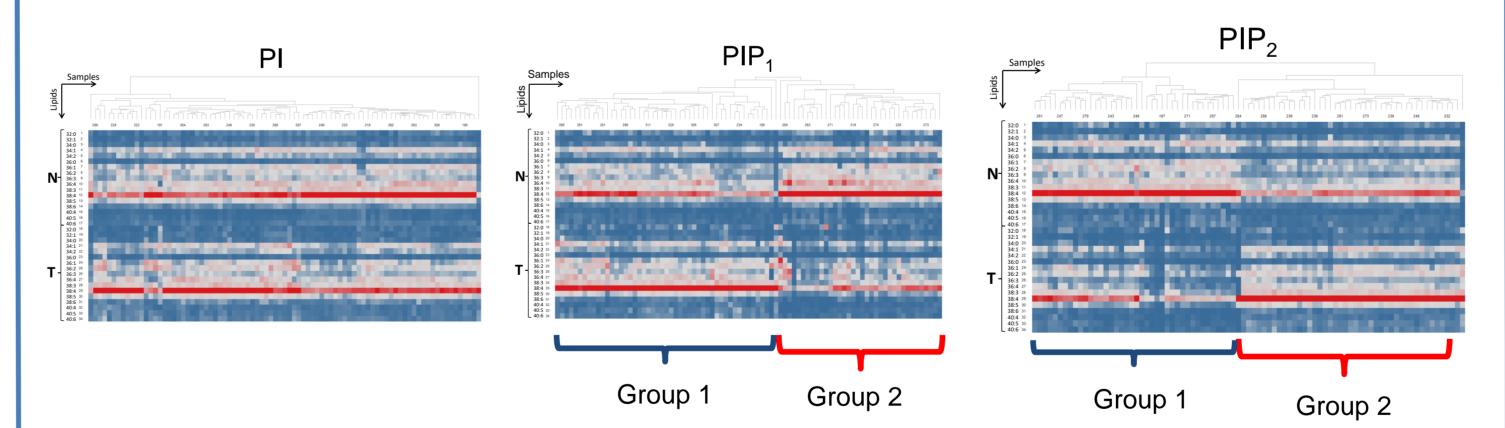






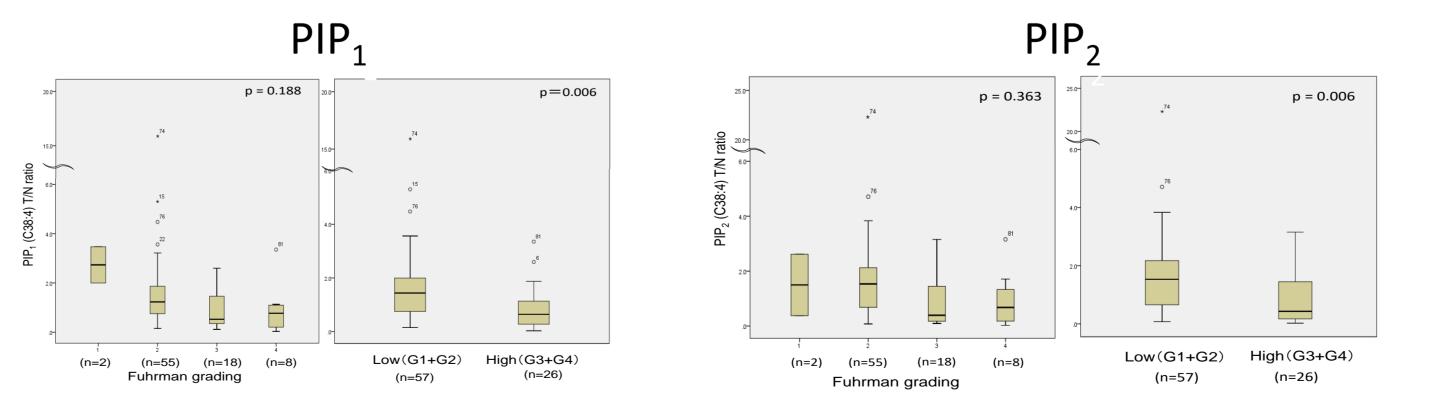
The PIP₁ (C38:4) T/N ratio was strongly correlated with the PIP₂ (C38:4) T/N ratio.

Fig. 3 Hierarchical cluster analysis of PIPs levels in all samples



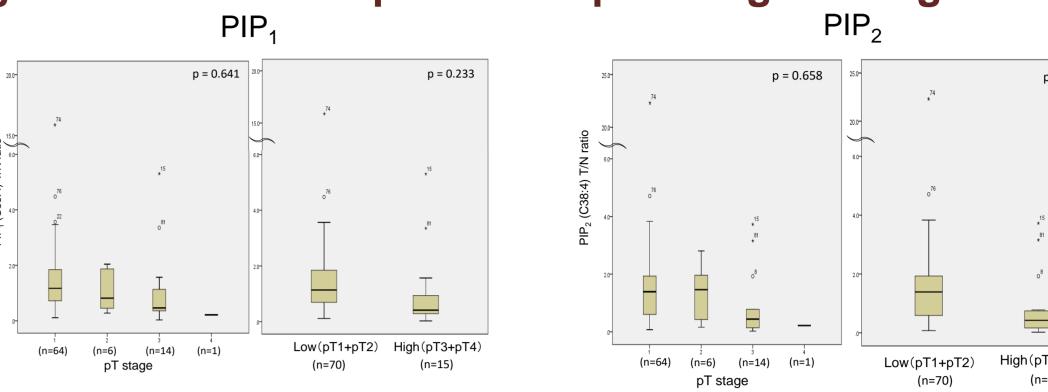
The PIP_1 and PIP_2 profiles can be dichotomized into two groups based on the difference of the levels between the tumor and normal surrounding renal tissue.

Fig. 4 The relationship between histological grade and PIPs levels



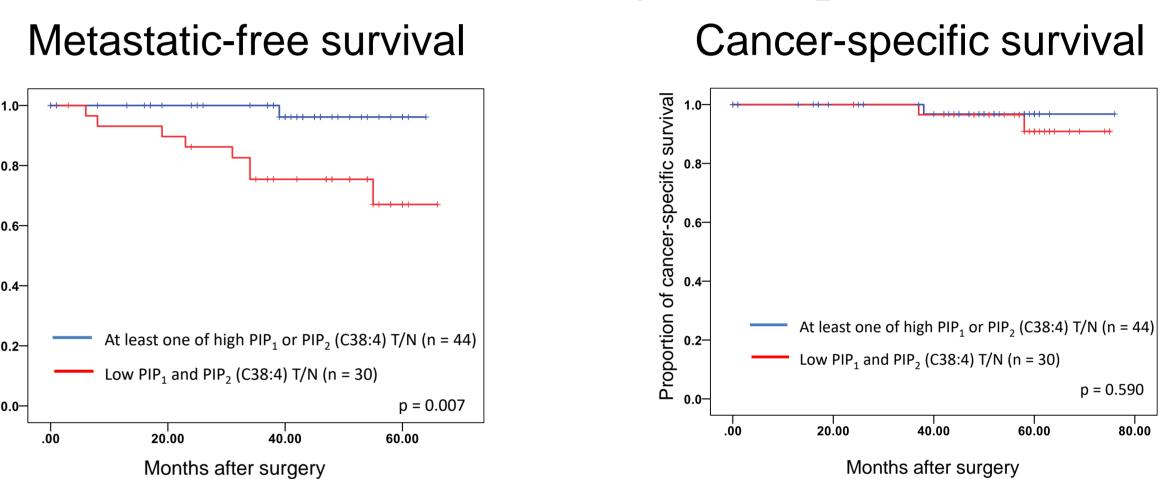
The patients with high grade tumor had significantly lower PIP₁ and PIP₂ (C38:4) T/N ratio.

Fig. 5 The relationship between pathological stage and PIPs levels



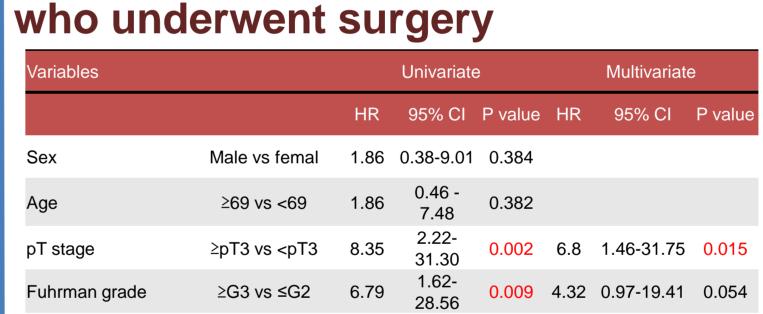
The patients with high stage tumor tended to have lower PIP₂ (C38:4) T/N ratio.

Fig. 6 Kaplan-Meier curves for survivals in patients with RCC who underwent surgery according to PIP₁ and PIP₂ (C38:4) T/N ratio



The 5-year metastatic-free survival in patients with low PIP_1 and PIP_2 (C38:4) T/N ratio was 67.0%, whereas that in patients having at least one of high PIP_1 and/or PIP_2 T/N ratio was 96.2%.

Table 3 Cox proportional hazards for MFS in patients with RCC



In multivariable analysis, a pT stage and a low PIP₁ and PIP₂ T/N ratio were independent prognostic factor for poor MFS in patients with RCC who underwent surgery.

PIP (C38:4) T/N

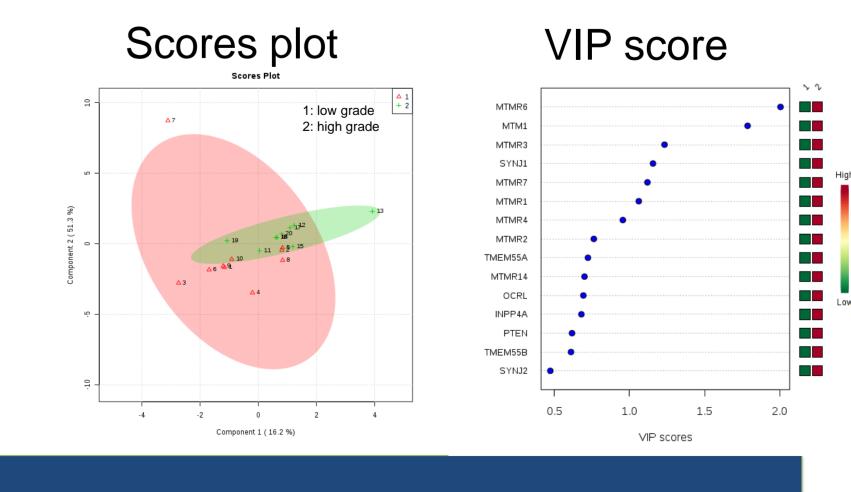
Low PIP₁ and PIP₂ vs others 10.2 1.27-81.84 0.029 9.23 1.01-77.53 0.041

Table 4 Correlation between *MTM1* and *MTMR6* mRNA and PIPs T/N ratio in patients with RCC treated with surgery

	MTM1 mRNA T/N		MTMR6 mRNA T/N	
	r ²	p value	r ²	p value
PIP ₁ (C38:4)T/N	0.619	0.005	0.591	0.010
PIP ₂ (C38:4)T/N	0.623	0.004	0.628	0.005
PI (C38:4)T/N	0.353	0.139	0.280	0.261

Among those phosphatases, the T/N ratios of MTM1 and MTMR6 mRNA were highly correlated with PIP₁ and PIP₂ (C38:4) T/N ratio.

Fig 7. PLS-DA analysis for the discrimination of low and high grade RCC according to 16 candidate phosphatases in patients with RCC treated with surgery



Scores plot and VIP scores showing the separation between low and high grade RCC using the T/N ratio of mRNA in 16 candidate phosphatases.

COI Disclosure

Shintaro Narita

The presenting author had no COI to disclose.

Conclusions

- The low PIP1 T/N and PIP2 (C38:4) T/N ratios were associated with cancer aggressiveness and poor metastatic-free survival in patients with RCC who underwent surgery.
- > PIPs levels in surgical specimen have a potential to be a biomarker for oncological outcome in patients with RCC, and targeting related enzymes of PIPs may become a novel treatment strategy for RCC.