MP08-14

Metabolomic approach to elucidate mechanisms of acquired resistance to sunitinib in renal cell carcinoma

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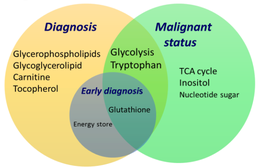
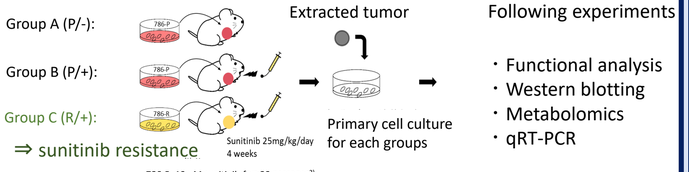
Introduction

- We identified characteristic metabolites and their pathways in renal cell carcinoma (RCC)from our previous global-metabolomics study.[1)]
- These metabolites are thought to be involved in resistance to sunitinib in RCC.
- The present study aimed to determine possible mechanisms of resistance to sunitinib, as well as the intracellular metabolites involved and the signaling pathways that regulate them.



T.Sato, International Journal of Cancer, 2019;145:

Materials and Methods

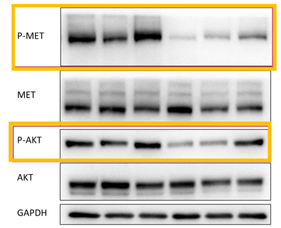


786-R: 10 μM sunitinib for>20 passages[2)]

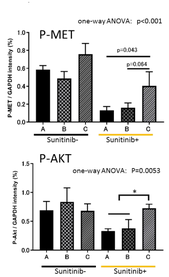
- 786-O RCC cells that had been exposed (786-R: sunitinib resistance) or not (786-P : control) to sunitinib were injected for nude mice, and then administered sunitinib to the mice for four weeks.
- Sunitinib-resistant (Group C) and control RCC cell lines were established from tumor extracted from these mice.
- We performed liquid chromatography-mass spectrometry to quantify the metabolites identified in our previous study and compare intracellular metabolism between the two cell lines (Group B vs Group C).

Results

1.Signal transduction pattern in sunitinib-resistant cells by Western blotting

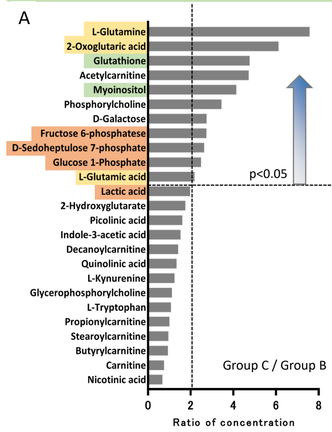


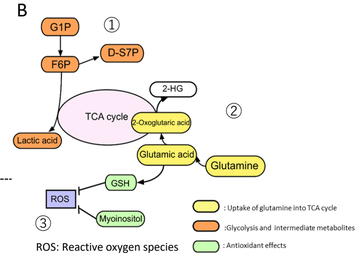
Group	A	B	C	A	B	C
Sunitinib	−	−	−	+	+	+



In the absence of sunitinib, phosphorylation of Akt and MET was increased in all three groups. In the presence of sunitinib, phosphorylation of Akt and MET was suppressed in group A (P/-) and group B (P/＋), but unsuppressed phosphorylation was observed in group C (R/＋).

2. Identification of metabolites that are upregulated in sunitinib-resistant cells



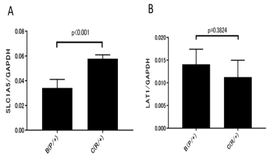


B) Metabolites and pathways increased with sunitinib-resistance
① Glycolysis and its intermediate metabolites
② Uptake of glutamine in the TCA cycle for energy metabolism
③ Antioxidant effects for reactive oxygen species (ROS).

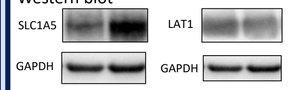
A) Eleven metabolites were significantly increased in group C (R/+), with a more than 2- fold increase in metabolite concentrations as compared to group B (P /+).

3. Expression of SLC1A5 was significantly increased in sunitinib-resistant cells

RT-PCR



Western blot

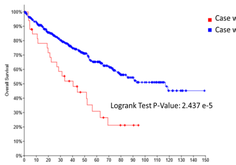


SLC1A5: a transporter that carries glutamine into cells
LAT1: a transporter that excrete glutamine from cells.

The expression of SLC1A5 was significantly increased in group C (R / +) . The expression of LAT1 was not significantly different between the two groups.

4. High SLC1A5 mRNA expression correlated with poor overall survival in ccRCC

Bioinformatics data -TCGA-



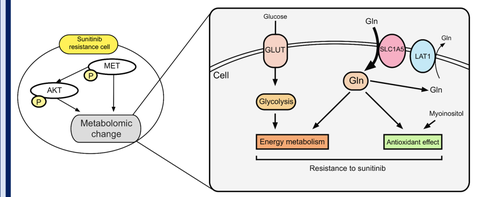
	Number of Cases, Total	Number of Cases, Deceased	Median Months Survival
Case with mRNA High in SLC1A5	34	22	40.4
Case without alteration mRNA in SLC1A5	478	148	116.8

In clear cell RCC, patients with mRNA high in SLC1A5 (n = 34) had poor overall survival in comparison to patients without alteration mRNA in SLC1A5 (p=0.00002)

Conclusion

- Energy metabolism with glutamine uptake and glycolysis upregulation, as well as antioxidant activity, contributed to the mechanism of sunitinib-resistance in RCC cells.
- Unsuppressed AKT and MET phosphorylation under sunitinib is associated with these metabolic pathways, and the glutamine transporter SLC1A5 is involved in sunitinib resistance.

Discussion



- Glutamine, a non-essential amino acid, is carried into cells by solute carrier (SLC)-type transporters, and one of these transporters, SLC1A5, is highly expressed in cancer cells.[3)]
- It was previously reported that V-9302, a competitive small molecule antagonist of SLC1A5, attenuates cancer cell growth and induces apoptosis of cancer cells by increasing oxidative stress. [4)]
- Thus, suppression of glutamine uptake via SLC1A5 could serve as a molecular target in the treatment of sunitinib-resistant RCC.

Reference

1) Sato, T. et al.: Value of global metabolomics in association with diagnosis and clinicopathological factors of renal cell carcinoma. Int J Cancer, **145:** 484, 2019
2) Hatakeyama,H. et al.: Investigation of Metabolomic Changes in Sunitinib-Resistant Human Renal Carcinoma 786-O Cells by Capillary Electrophoresis-Time of Flight Mass Spectrometry. Biol Pharm Bull, **41:** 619, 2018
3) Hoerner, C. R. et al.: The 'Achilles Heel' of Metabolism in Renal Cell Carcinoma: Glutaminase Inhibition as a Rational Treatment Strategy. Kidney Cancer, **3:** 15, 2019
4) Schulte, M. et al.: Pharmacological blockade of ASCT2-dependent glutamine transport leads to antitumor efficacy in preclinical models. Nat Med, **24:** 194, 2018