Comprehensive analysis of the metabolic enzymes in patients with small cell lung cancer using a large-scale targeted proteomics assay

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Abstract

Background: Small-cell lung cancer (SCLC) is a devastating subtype of lung cancer, and its biological, clinical and genetic characteristics differ from those associated with non-small-cell lung cancer. Despite the comprehensive genetic analysis of SCLC, promising therapeutic targets have yet to be identified.
Methods: We applied *in vitro* proteome-assisted multiple reaction monitoring for protein absolute quantification (iMPAQT), which can allow absolute quantification of 342 metabolic enzymes. Frozen samples obtained from 36 patients with surgically resected SCLC (n = 12), adenocarcinoma (ADC; n = 12) and squamous cell carcinoma (SCC; n = 12) were analyzed by iMPAQT. An enrichment analysis was used to identify metabolic pathways specifically varying in SCLC. *P*-value of <0.05 were considered to indicate statistical significance.
Results: The iMPAQT analysis revealed that the purine metabolic pathway was the most upregulated pathway among the metabolic pathways that specifically vary in SCLC ($P = 1.1 \times 10^{-10}$). Among enzymes associated with purine metabolism, only hypoxanthine guanine phosphoribosyltransferase 1 (HPRT1), a key enzyme for the salvage pathway of purine metabolism, was significantly overexpressed in SCLC in comparison to ADC and SCC ($P = 2.0 \times 10^{-6}$). The higher expression of HPRT1 was significantly associated with a larger tumor size ($P = 2.0 \times 10^{-3}$) and poorer overall survival in patients with SCLC ($P = 7.9 \times 10^{-3}$).
Conclusions: The current study showed that purine metabolic enzymes, especially HPRT1, were significantly upregulated in SCLC in comparison to ADC and SCC. Furthermore, the absolute amount of HPRT1 was larger in SCLC patients with clinically malignant traits, suggesting that HPRT1 may be associated with the tumorigenesis of SCLC and contribute to the acquisition of malignant traits.

Objective

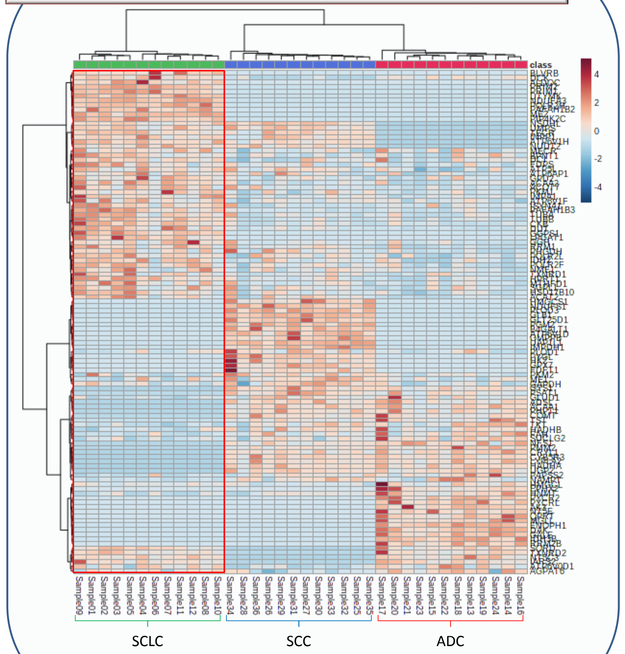
The purpose of this study was to identify metabolic enzymes which may contribute to malignancy in small cell lung cancer (SCLC) using *in vitro* proteome-assisted multiple reaction monitoring for protein absolute quantification (iMPAQT), which comprehensively allows absolute quantification of metabolic enzymes.

Materials and methods

- Frozen samples obtained from 12 SCLC (6 corresponding normal tissues), 12 squamous cell carcinoma (SCC) and 12 adenocarcinoma (ADC) patients, who were resected at Kyushu University between 2012 and 2018, were used in this study.
- Smoker and male sex were frequently observed in SCLC and SCC as compared with ADC, which might reflect the clinical features of each histology (data not shown).
- iMPAQT was developed to allow absolute quantification of metabolic enzymes and in this study, the method was performed by Kyushu Pro Search LLP.

Matsumoto M, et al., *Nat Methods*, 2017;14:251-8
Kodama M, et al., *Nat Commun*, 2020;11:1320

Heat map for hierarchical clustering of metabolic enzymes by iMPAQT in 36 frozen samples obtained from 12 SCLC, 12 SCC and 12 ADC patients



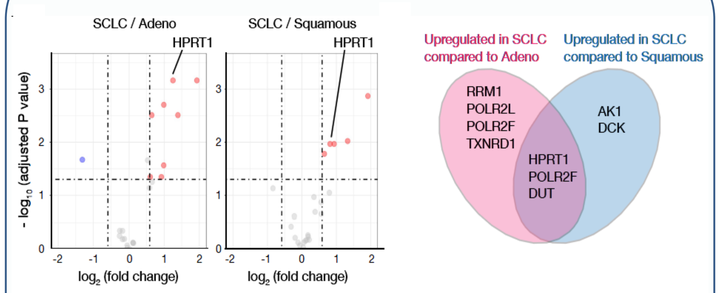
The iMPAQT analysis revealed a definitive difference in the absolute amount of the metabolic enzymes between SCLC, ADC and SCC.

KEGG pathway enrichment analysis for various metabolic pathways

KEGG_PATHWAY	PValue
hsa00230:Purine metabolism	1.1E-10
hsa00240:Pyrimidine metabolism	2.6E-10
hsa00280:Valine, leucine and isoleucine degradation	6.0E-06
hsa00520:Amino sugar and nucleotide sugar metabolism	7.0E-06
hsa00062:Fatty acid elongation	3.1E-05
hsa00052:Galactose metabolism	7.9E-05
hsa00071:Fatty acid degradation	4.1E-04
hsa00051:Fructose and mannose metabolism	1.3E-03
hsa00500:Starch and sucrose metabolism	1.5E-03
hsa00190:Oxidative phosphorylation	4.1E-03
hsa00900:Terpenoid backbone biosynthesis	4.3E-03
hsa01040:Biosynthesis of unsaturated fatty acids	4.9E-03
hsa00650:Butanoate metabolism	7.7E-03
hsa00920:Sulfur metabolism	7.7E-03
hsa00310:Lysine degradation	7.9E-03
hsa00760:Nicotinate and nicotinamide metabolism	9.5E-03
hsa00072:Synthesis and degradation of ketone bodies	9.6E-03
hsa00010:Glycolysis / Gluconeogenesis	1.9E-02
hsa00620:Pyruvate metabolism	2.3E-02
hsa00450:Selenocompound metabolism	2.7E-02
hsa00480:Glutathione metabolism	4.2E-02
hsa00514:Other types of O-glycan biosynthesis	4.4E-02

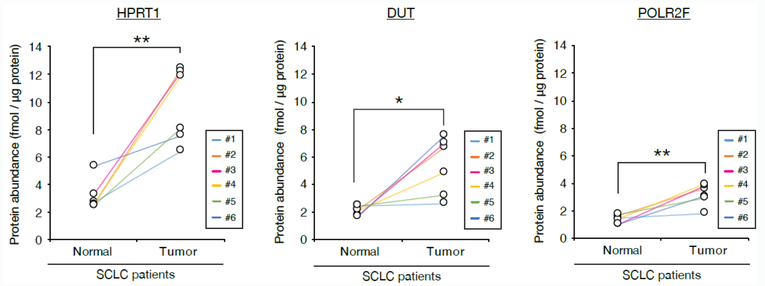
KEGG pathway enrichment analysis showed that the purine metabolic pathway was the most upregulated one that specifically varied in SCLC patients ($P = 1.1 \times 10^{-10}$).

Comparison of the protein amount of metabolic enzymes between SCLC and ADC/SCC



HPRT1, POLR2F and DUT were commonly upregulated in SCLC as compared with SCC and ADC.

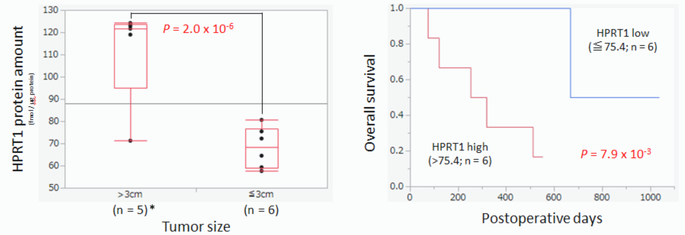
Differences of HPRT1, DUT and POLR2F protein amount between tumor and corresponding normal tissues in 6 SCLC patients



*: P < 0.05; **: P < 0.01

Differences of HPRT1 protein amount between tumor and corresponding normal tissues were larger than those of DUT and POLR2F.

Association between HPRT1 expression levels and tumor size and postoperative overall survival



* The information on tumor size in one patient was unavailable.

The higher expression of HPRT1 was significantly associated with a larger tumor size ($P = 2.0 \times 10^{-3}$) and poorer overall survival in 12 patients with resected SCLC ($P = 7.9 \times 10^{-3}$).

Conclusions and discussions

- The iMPAQT analysis revealed that the purine metabolic pathway was the most upregulated one among the metabolic pathways that specifically varied in SCLC ($P = 1.1 \times 10^{-10}$).
- HPRT1, POLR2F and DUT were significantly upregulated in SCLC compared to SCC and ADC; however, differences of HPRT1 protein amount between tumor and corresponding normal tissues were larger than those of DUT and POLR2F.
- The higher expression of HPRT1 was significantly associated with a larger tumor size ($P = 2.0 \times 10^{-3}$) and poorer overall survival in 12 patients with resected SCLC ($P = 7.9 \times 10^{-3}$).
- **Further preclinical and clinical analyses are warranted to elucidate the exact significance of purine metabolic pathway, especially HPRT1, in the tumorigenesis, growth and survival of SCLC.**

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COI statement

None of the authors declare any conflicts of interest.