Proteomics Study on the Effect of General Anesthesia on Human Plasma and Peripheral Blood Mononuclear Cells

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Introduction

General anesthesia, a commonly used procedure before surgery of serious diseases such as cancers, may be an under-evaluated pre-analytical factor that impacts biomarker studies. The biochemical mechanism of action of general anesthetics is not yet well understood. To induce unconsciousness, anesthetics have myriad sites of action and affect the central nervous system (CNS) at multiple levels. Thus a great number of research studies on general anesthesia effects primarily focus on CNS and neural networks whose interruption are linked with unconsciousness. Nevertheless, only a few studies on effect of anesthesia have been conducted on body fluids/tissues/organs other than CNS. Therefore, we performed an in-depth, unbiased proteomics study to assess the effect of general anesthesia on human plasma and peripheral blood mononuclear cells (PBMC).

Methods

Samples were collecting from 40 rectum and sigmoid colon cancer patients undergoing surgery and anesthetized by inhalation of Desfluran or Sevofluran/Sevorane. The whole blood samples were drawn before and after anesthesia, collected in EDTA tubes and poured into a blood separation tube containing the ficoll or lymphocyte separation medium (PAA, Cölbe, Germany, item number J15-004). After the blood tube was centrifuged, plasma from the top layer was collected and frozen at -80°C. Subsequently, the PBMC fraction was taken after removal of plasma. The PBMC cells were washed by PBS and counted and frozen.

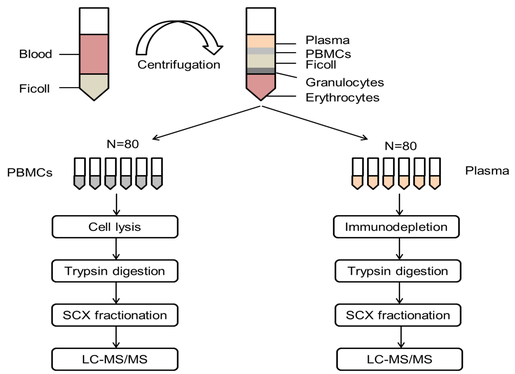


Figure 1. Schematic overview of the PBMC and plasma sample preparation method.

The high abundant proteins in plasma were firstly removed using MARS-14 depletion. PBMC cells were lysed in cold lysis buffer. Then proteins from plasma and PBMC cells were reduced by TCEP, digested with trypsin, pre-fractionated offline by SCX chromatography and analyzed by a high resolution Q Exactive mass spectrometer coupled to a nano flow LC for both protein qualification and quantification simultaneously.

For peptide and protein identification, all MS/MS spectra were searched against human database Swissprot using Mascot (Matrix Science, London, UK) which was integrated into Elucidator (Rosetta Biosciences) .

For statistical analysis, all peak intensities were log transformed and normalized. To compare before to after anesthesia on peptide level, the following ANOVA model was used: $I_{ijkl} = M + T_i + P_j + C_k + \varepsilon_{ijkl}$
where I was the measured peptide intensity, M was the overall average intensity, T was the 'type' factor (before or after anesthesia), P was the 'patient' and C is the peptide/transition factor . The number of the levels for C is protein-dependent, equal to the number of children peptides for the protein.

Results

One of the largest human PBMC proteome profiles reported thus far : 4,382 unique proteins identified and quantified, representing 39,303 peptides from all 4 SCX fractions in human PBMCs with the FDR cutoff 1%. There were overlapped peptides among 4 SCX fractions. However, a majority of peptides were unique to each fraction.

	TOTAL ALL FRACTIONS	TOTALS			
		In Fraction 1	In Fraction 2	In Fraction 3	In Fraction 4
#Peptides	39,303	10,962	12,900	14,132	13,589
#Proteins	4,382	2,494	3,135	2,842	2,221

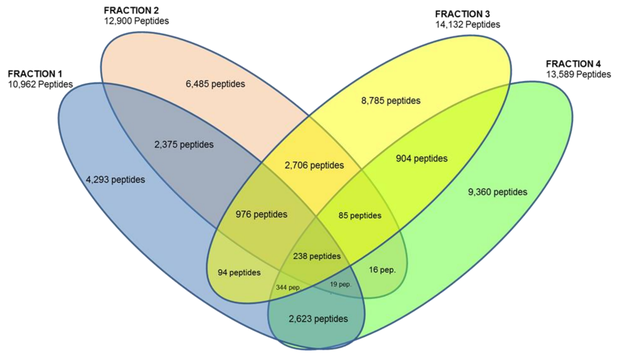


Figure 2. The numbers of identified proteins and peptides in total and each of 4 SCX fractions in PBMCs and Venn diagram showing the unique and overlapped peptides in 4 fractions.

76% of identified proteins had two or more peptides sequenced per protein, which meant the identification had high confidence

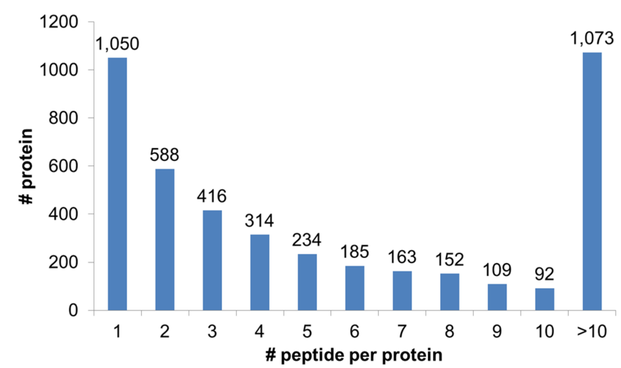


Figure 3. Distribution of identified proteins in PBMCs with different number of peptides per protein.

Results

Samples were very well correlated to each other. Overall quantitative results indicated that very few changes were observed in either PBMCs or plasma after anesthesia administration.

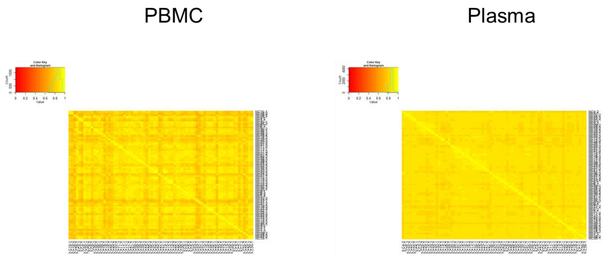


Figure 4. Pearson correlation map of all the human PBMC and plasma samples before and after anesthesia. Intensities of molecular ions after normalization were used to draw the map.

No distinguishable differences between before and after anesthesia samples

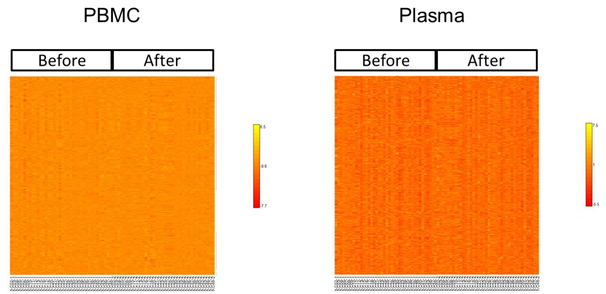


Figure 5. Heatmap of all identified proteins from human PBMC and plasma before and after anesthesia administration. The y-axis z-score is calculated for a protein x as (abundance x – abundance mean) / abundance standard deviation.

9 proteins out of the total 4,382 proteins were significantly changed in PBMCs and only 1 protein out of 621 plasma proteins was changed upon anesthesia administration

PBMC

		After vs Before				
UniProt_ID	Gene	DE	DI	p-Value	q-Value	Protein Description
TPOR_HUMAN	MPL	X	2.61	0.000	0.002	Thrombopoietin receptor precursor
MYADM_HUMAN	MYADM	X	2.20	0.000	0.000	Myeloid-associated differentiation marker
DNJA3_HUMAN	DNAJA3	X	2.05	0.001	0.009	DnaJ homolog subfamily A member 3, mitochondrial precursor
UBE4B_HUMAN	UBE4B	X	1.71	0.002	0.015	Ubiquitin conjugation factor E4 B
APOC2_HUMAN	APOC2	X	1.62	0.000	0.000	Apolipoprotein C-II precursor
TTPA_HUMAN	TTPA	X	1.55	0.006	0.039	Alpha-tocopherol transfer protein
PUR1_HUMAN	PPAT	X	1.51	0.003	0.024	Amidophosphoribosyltransferase precursor
CLC4A_HUMAN	CLEC4A	X	0.59	0.006	0.036	C-type lectin domain family 4 member A
TXN4B_HUMAN	TXNL4B	X	0.60	0.000	0.001	Thioredoxin-like protein 4B

Plasma

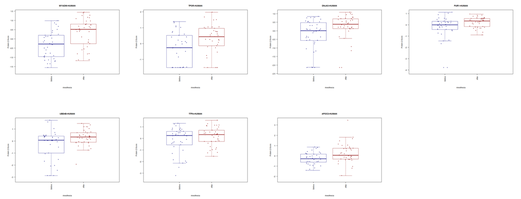
		After vs Before				
UniProt_ID	Gene	DE	DI	p-Value	q-Value	Protein Description
PRL_HUMAN	PRL	X	5.91	0.000	0.000	Prolactin

Figure 6. Differentially expressed (DE) proteins by comparing after to before anesthesia in human PBMC and plasma samples. The significance criteria for PBMC are p-value < 0.05, q-value < 0.05, fold change (DI) > 1.5. The significance criteria for plasma are p-value < 0.05, q-value < 0.05, fold change (DI) > 2.

Results

Among the 9 DE proteins in PBMCs, 7 proteins were up-regulated while 2 were down-regulated upon anesthesia administration

Up-regulated



Down-regulated

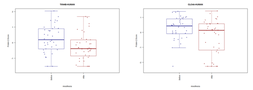


Figure 7. Box plots of z-score of 9 differentially expressed protein after anesthesia administration in human PBMC samples. Among them, 7 proteins are up-regulated and 2 are down-regulated.

Six proteins (DNAJA3, MPL, PPAT, TTPA, TXNL4B, and UBE4B) out of 9 were annotated as necrosis related. Three proteins (DNAJA3, MPL, PPAT) were associated with proliferation of blood cells. Three proteins (CLEC4A, MPL, PPAT) were linked to immunological disease.

Diseases or Functions Annotation	p-Value	Molecules	# Molecules
necrosis	1.80E-03	DNAJA3,MPL,PPAT,TTPA,TXNL4B,UBE4B	6
proliferation of blood cells	1.16E-02	DNAJA3,MPL,PPAT	3
Immunological Disease	2.37E-02	CLEC4A,MPL,PPAT	3
cell death of hematopoietic progenitor cells	5.83E-03	DNAJA3,MPL	2
proliferation of hematopoietic progenitor cells	7.17E-03	DNAJA3,MPL	2
development of lymphoid organ	7.38E-03	DNAJA3,MPL	2
proliferation of T lymphocytes	4.62E-02	DNAJA3,PPAT	2

Figure 8. Top enriched functions of 9 differentially expressed proteins after anesthesia administration in human PBMC sample.

Conclusions

The two dimensional LC fractionation coupled to label-free LC-MS/MS quantification platform enables unbiased and in-depth comparison of the proteomes of human PBMC and plasma upon general anesthesia administration. A few changed proteins in PBMCs are new findings and can be linked to previously reported biological effects of anesthesia. Using rigorous statistical analysis, this study demonstrates that most proteins remain unchanged and that anesthesia, as a pre-analytical variable, does not affect the proteomes of PBMC and plasma significantly. But caution must be used to interpret results in the future proteomics studies when encountering differentially expressed proteins reported herein. Furthermore, it would be interesting to apply this platform to conduct studies on effect of anesthesia on CSF protein integrity or metabolites.

Acknowledgements

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