Description of case-control investigation of ovarian cancer

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1 Experimental design

The investigation aimed at finding proteins that systematically change in abundances between plasma samples of patients with epithelial ovarian cancer and healthy controls, thereby focusing on N-glycosylated proteins. It was conducted in a case-control experimental design, with six disease and ten control patients. The group of 14 discriminatory proteins was represented by 1-2 peptides, and each peptide was represented by 2-3 transitions.

2 Sample selection

Sample selection Plasma samples were prospectively collected at the Department of Gynaecology at University Hospital Zürich after written informed consent was given. Ethical approval for this study was granted by the appropriate Ethical Board in 2006 (SPUK, Canton of Zürich, Switzerland). Plasma samples were collected at primary diagnosis immediately prior surgery from healthy control patients with subsequently proven negative intra-operative findings (n=10) and epithelial ovarian cancer patients (n=6). Patients with a past history of cancer or with chronic infectious diseases of non-gynaecological and gynaecological origin were excluded from the study.

3 Sample preparation

Two venous blood samples (12ml) were collected per patient in EDTA blood tubes (BD Vacutainer, 0.184M EDTA, BD Diagnostics, Franklin Lakes, US) and stored on ice for a maximum of three hours until further processing. Blood samples were centrifuged at 3000g for ten minutes at 4°C, and aliquots of the supernatant plasma frozen at -80°C. Specimen were subjected to N-glycoprotein enrichment[1] and trypsin digestion. Heavy-labeld AQUA[2] peptides were spiked into each plasma sample as an internal reference before the MS analysis.

4 Mass spectrometric analysis

SRM transitions were optimized for each glycopeptide using AQUA peptides, and collision energy was optimized for all transitions as described in (Picotti et. al., 2010). For each peptide, three transitions with the best signal-to-noise ratio were selected for the quantitative analysis. The order of the biological samples was randomized, and each sample was profiled in a single MS run, with no technical replicates. Quantitative measurements were performed on 4000 QTRAP instrument in time-scheduled SRM mode using software Analyst 1.5 (Applied Biosystems/MDS Sciex), and peptide separation using Tempo Nano LC system (Applied Biosystems/MDS Sciex).

5 Signal processing

Each transition was quantified by its peak apex automatically, using MultiQuant software package (Applied Biosystems/MDS Sciex). The quantified transitions were manually curated, resulting in the total of 44 quantified transitions and a small number of missing values.

1. Zhang, H., Li, X., Martin, D. & Aebersold, R. Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. *Nature Biotechnology* **21**, 600–666 (2003).

2. Gerber, S. A., Rush, J., Stemman, O., Kirschner, M. W. & Gygi, S. P. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *Proceedings of the National Academy of Science of the USA* **100**, 6940–6945 (2003).