

Glyco-centric serum proteomics applied to Barrett's esophagus and esophageal adenocarcinoma

Alok K. Shah¹, Vojtech Franc², Wioleta Zelek³, Gunter Hartel¹, Ian Brown⁴, Clay Winterford¹, Bradley Spicer⁵, Michelle Dunstone⁵, PROBE-NET⁶, Virendra Joshi⁷, David Whiteman¹, B Paul Morgan³, Albert J. R. Heck², and Michelle M. Hill¹

¹QIMR Berghofer Medical Research Institute, Australia; ²University of Utrecht, The Netherlands; ³Cardiff University School of Medicine, UK; ⁴Envoi Pathology, Australia; ⁵Monash University, Australia; ⁶Centre for Research Excellence, Australia; ⁷Ochsner Health System, USA

Introduction: There is an unmet clinical need for first-line blood biomarker based surveillance tool to monitor Barrett's esophagus (BE) patients for development of esophageal adenocarcinoma (EAC). Earlier, we discovered serum glycoprotein biomarker candidates that differentiate between BE and EAC phenotype (1). To progress biomarker development, we conducted independent cohort evaluation of biomarker candidates, including dysplastic condition. In addition, we selected the top candidate protein, complement component C9 (C9), for tissue immunohistochemistry and serum protein proteoform and glycopeptide analyses.

Methods: 301 serum samples were analyzed using previously established targeted glyco-centric proteomics workflow (1). Univariate (ROC curve) and multivariate (recursive partitioning) statistical analyses were performed. C9 was purified from pooled serum samples of different disease groups and subjected to proteoform analysis (2).

Results: C9 and gelsolin were validated as top diagnostic biomarker candidates for EAC (3). A panel of 10 serum glycoprotein candidates, which includes 6 complement pathway proteins showed high discriminatory value (AUROC 0.93) to select BE patients requiring intervention (high grade dysplasia and EAC). Immunohistochemistry on esophageal tissues confirmed elevated C9 protein during progression through BE/EAC. However, initial serum C9 proteoform and glycopeptide analysis of pooled samples showed only minor differences in C-mannosylation and O-linked sialylation in EAC.

Conclusions: Glyco-centric proteomics facilitated discovery and validation of serum biomarker candidates for BE surveillance. Potential alterations in complement pathway function merit further clinical and molecular validation.

1. Shah, A. K., et al. 2015 *Mol Cell Proteomics* 14; 3023-3039
2. Franc, V., et al. 2017 *Anal Chem* 89; 3483-3491
3. Shah, A. K., et al. 2018 *Mol Cell Proteomics* 17: 2324-2334