Secretome-based Proteomic Strategies for Uncovering New Biomarkers for Colorectal Cancer

Joint ProteomicS Laboratory (JPSL), Ludwig Institute for Cancer Research & The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, PO Box 2008, Parkville, Victoria 3050, Australia Richard.Simpson@ludwig.edu.au

Colorectal cancer (CRC) is a leading cause of cancer death in the Western World. Early detection is the single most important factor influencing outcome of CRC patients. If identified while the disease is still localized CRC is treatable. To improve outcomes for CRC patients there is a pressing need to identify biomarkers for the early detection (diagnostic markers), prognosis (prognostic indicators), tumor responses (predictive markers) and disease recurrence (monitoring markers). Despite recent advances in the use of genomic analysis for risk assessment, in the area of biomarker identification genomic methods have yet to produce reliable candidate markers for CRC. For this reason, attention is now being directed towards protein chemistry or proteomics as an analytical tool for biomarker identification. Here, we discuss an integrated proteomic/genomic strategy directed towards CRC biomarker discovery. This strategy involves the proteome analysis of a panel of CRC cell lines focusing on the secretome, which comprises soluble secreted proteins including glycoproteins and low-Mr polypeptides. We describe a reverse-engineering approach for identifying potential CRC biomarkers and a strategy for validating our in vitro findings using an in vivo mouse model.
Cell-based Proteome Analysis: The First Stage in the Pipeline for Biomarker Discovery

Barbara Sitek¹, Gereon Poschmann¹, Corinna Henkel¹, Bence Sipos², Oliver Vonend³, Helmut E. Meyer¹ and K. Stühler¹

¹Medizinisches Proteom-Center, Ruhr-University Bochum, Germany
²Department of Pathology, University of Kiel, Germany
³Department of Nephrology, Marienhospital Herne, Ruhr-Universität Bochum, Germany

Introduction Until now, proteomics as a technology allowing quantifying and identifying proteins within complex mixtures could not significantly contribute to the field of biomarker discovery. Therefore, we established a cell-based proteomics approach allowing us to identify candidate biomarkers with a high success rate in the verification phase. Here, we will outline our approach of cell specific proteome analysis as the first decisive step in the pipeline for new diagnostics applying high sensitive protein detection method for the analysis of scarce amount of sample.

Methods In order to identify new molecular markers for e.g. pancreatic intra-epithelial neoplasias (PanINs), nephron glomeruli or liver cirrhosis, we established a proteomics approach analysing microdissected cells. Due to the limited amount of proteins available from microdissection (1000 cells) we developed a procedure including fluorescence dye saturation labelling in combination with high resolution two-dimensional gel electrophoresis (2-DE). For the differential proteome analysis by the 2D DIGE technique 2-5 μg per cell lysate were labelled with fluorescence dyes and separated by 2D electrophoresis. For protein identification MALDI-TOF-MS and nanoLC-ESI mass spectrometer was used.

Results By applying microdissection combined with saturation DIGE technique and mass spectrometry we could succeed in finding new biomarker candidates for liver cirrhosis, pancreatic intra-epithelial neoplasias (PanINs) and nephron glomeruli. This technique allows us to analyze quantitatively the proteome of just a few thousand cells from individual patient samples and to get reproducible proteomics data pointing to new biomarker candidate proteins for liver cirrhosis. Verification of these candidate biomarkers could be demonstrated by immunohistochemistry. By Western blotting first candidate biomarker could be identified in sera of patients with late stage liver fibrosis of different genesis.

Innovative aspects
- Cell-based proteomics approach allows identifying candidate biomarkers with a high success rate in the verification phase.
Mechanisms underlying disease pathogenesis are not well understood in the context of common etiological factors such as microbial infection, inflammation, malignancy or tissue breakdown. Such processes may be elucidated by identifying disease-related molecular markers, such as acute phase proteins, cytokines, cytoskeletal fragments and autoantigens. In an attempt to identify such markers, we used two proteomic methods to analyze plasma samples from patients and healthy donors. The first method allowed to evaluate changes in concentration of glycoproteins, and to comprehensively survey the plasma proteome. The second method, intact peptidomics, was used to assess changes in endogenous proteolytic activity by analyzing the low molecular weight (LMW) component. The integrated proteomic and peptidomic analysis of plasma samples identified a number of cytoskeletal and Ca\(^{2+}\)-binding proteins and their proteolytic fragments in the disease samples. The measurements were compared to healthy donors and several of the observed differential quantitations were independently verified by ELISA. The identified changes in plasma proteome and peptidome, and the underlying altered endogenous protease activity may result in the generation of novel autoantigens. We have confirmed this hypothesis by the observation of autoantibodies in patients and upon extension of these studies to larger populations of patients; we may gain additional understanding of the role of etiological factors in different disease pathways and genomic penetrance.
Shotgun proteomics of human gastric juice

Maxey C.M. Chung1,2, Cynthia R.M.Y. Liang2, Qingsong Lin2, Sandra Tan2, Teck Kwang Lim2, Yi Liu2, Khay Guan Yeoh3, Jimmy So4 and Choy Leong Hew2
1Department of Biochemistry, Yong Loo Lin School of Medicine, 2Oncoproteomics Laboratory, Department of Biological Sciences, 3Department of Medicine, Yong Loo Lin School of Medicine, 4Department of Surgery, National University Hospital, Lower Kent Ridge Road, Singapore 119077

Introduction

Human gastric juice consists of 2.5L of fluid that is secreted daily by gastric glands and stomach surface epithelial cells. This fluid contains proteins that aid in digestion as well as homeostasis of the gastrointestinal (GI) tract. The gastric juice proteome represents a potential reservoir of biomarkers for diagnosis of stomach diseases as it is the proximal most fluid bathing the tissue. Global analysis of gastric proteome will also aid in understanding GI health and disease pathogenesis. Here, we employed shotgun proteomics to establish a database of the human gastric juice proteome.

Methods

Gastric juice from patients with chronic gastritis was collected during endoscopy after an overnight fast. Only clear, straw-coloured samples were analyzed. Tris and protease inhibitors were immediately added upon collection. 125μg of clarified protein was subjected to reduction and alkylation. Tryptic digest was then carried out at a trypsin:protein ratio of 1:30. The sample (duplicates) was then separated by reverse-phase capillary liquid chromatography (LC). Two LC separations using 8μg & 24μg protein were performed and the collected fractions were subsequently analyzed with a MALDI TOF/TOF mass spectrometer. Precursors selected for the MS/MS analysis of the 1st LC separation were excluded from the analysis of the 2nd LC run. The mass spectra were combined for database search using MASCOT against an IPI human database. (See Figure 1)

Results

Using 5% false positive rate as the peptide cut-off threshold, proteins were identified with at least two-peptide matches and false-positive identification at the protein level at 0. Proteins with single peptide matches were identified when using 0% false positive rate as the peptide cut-off threshold. With these criteria, the number of proteins identified among the 6 individual patients ranged from 90 to 170, with a mean of 136. Whilst technical replicates between runs in individual patients were highly reproducible, the biological reproducibility between patients was low. This resulted in a total of 355 proteins being identified from the 6 patients altogether, with 35% of the proteins being consistently identified in at least 3 out of 6 patients. Gene Ontology showed that 43% of all the proteins identified were secreted proteins. Our results reveal that the gastric juice proteome consists of resident gastric proteins such as mucins, intrinsic factor and pepsinogen. Additionally, salivary amylase, cystatins, immune-related proteins, α-1-acid glycoprotein, S100A family of proteins and enolase were also commonly identified among the gastric juice proteomes of these patients.

Innovative aspects

• First report of large scale profiling of human gastric juice proteome using LC-MS/MS
• Database of human gastric juice proteome containing resident gastric proteins as well as novel candidates
• Creates a useful reference for biomarker discovery in gastric disorders

References


Figure 1. LC-MS/MS workflow for gastric juice proteome profiling