Investigations into the Effects of Blood Specimen Handling Procedures on Protein Integrity

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Overview of project

1) Recruit 250 subjects of breast and prostate cancer at varying stages; single draw

2) Vary collection as well as plasma and serum preparation procedures in a controlled and randomized manner

3) Examine protein abundances and modifications (e.g. oxidation, proteolysis)

4) Use two technology platforms:
   Label-free liquid chromatography-mass spectrometry (LC-MS) differential expression
   Multiplexed Immunoassay (IA)

5) Correlate with other laboratory measures and patient information

6) Identify a panel of protein concentrations and modifications as a metric of sample integrity

7) Establish a targeted multiple-reaction monitoring (MRM) LC-MS method for this panel for efficient measurement and widespread use

8) Evaluate numerous existing collections using this panel

Research Team in addition to PPD

Patient Recruitment, Phlebotomy and Initial Collection Tube Manipulations

Palo Alto Medical Foundation (PAMF)

Arthur Bobrove, M.D., Director of Research

Multiplexed Immunoassay (IA) Development

Millipore Corp. (using Luminex xMAP Technology)

Jehangir Mistry, Ph.D.
Director, R&D, Bioscience Division

Linda Meeh, Ph.D.
Director, Protein Research Assay Group

Including resources from the former Linco and Upstate sites

Research Team at PPD

Final sample manipulations

LC-MS measurements

IA measurements

Statistics

Reporting

Additional key individuals:
      Hua Lin, Ph.D., Associate Director
      Ted Jones, Ph.D., Senior Biostatistician

Plasma and Serum collection variables

1) Number of subjects, 250; single draw.   N = 25 for individual variables

2) Blood collection tube type (5 varieties)

3) T_b= Blood time from draw to centrifugation (0.5 to 6 hours)

4) T_p= Plasma time from centrifugation to pipetting (& freezing) (0.5 to 6 hours)

5) T_f= Time in freezer (to 3 years, plus archived samples)

6) N_c= Number of freeze-thaw cycles (1 to 5)

Label-Free LC-MS Differential Profiling:

exemplary proteins from several hundred ID'd, ~ 5000 ID'd peptides

–Wang et al. Analytical Chemistry 75:4818-4826 (2003)

Actin, cytoplasmic 1 (Beta-actin)
Antithrombin-III (ATIII)
Apolipoprotein C-III (Apo-CIII) (ApoC-III)
Apolipoprotein E (Apo-E)
Biotinidase
Carboxypeptidase N catalytic chain (CPN) (Kininase-1)
Coagulation factor X (Stuart factor)
Coagulation factor XIII
Complement component C8
Complement factor H-related protein 1 (FHR-1) (H factor-like protein 1)
Corticosteroid-binding globulin (CBG) (Transcortin) (Serpin A6)
Extracellular matrix protein 1 (Secretory component p85)
Ficolin-3 (Collagen/fibrinogen domain-containing protein 3)
Gelsolin (Actin-depolymerizing factor) (ADF) (Brevin) (AGEL)
Glutathione peroxidase 3 (GSHPx-3) (GPx-3)
Heparin cofactor 2 (HC-II) (Protease inhibitor leuserpin 2) (HLS2)
Insulin-like growth factor-binding protein complex acid labile chain (ALS)
Lumican (Keratan sulfate proteoglycan lumican) (KSPG lumican)
Pigment epithelium-derived factor (PEDF) (Serpin-F1) (EPC-1)
Protein S (alpha)
Selenoprotein P (SeP)
Serum amyloid A-4 protein (Constitutively expressed serum amyloid A protein) (C-SAA)
Serum paraoxonase/arylesterase 1 (PON 1) (Serum aryldialkylphosphatase 1) (A-esterase 1)
Thyroxine-binding globulin (T4-binding globulin) (Serpin A7)
Vitronectin (Serum-spreading factor) (S-protein) (V75)

LC-MS Profiling of Modifications: primarily proteolysis and oxidation

Antithrombin-III (ATIII)
  FATTFYQH
  FATTFYQHLAD
  FATTFYQHLADSK
  NDNDNIFLSPL
  NDNDNIFLSPLSISTAF
  NDNDNIFLSPLSISTAFAMTK
  TSDQIHFF
  TSDQIHFFF
  TSDQIHFFFAK

Gelsolin (Actin-depolymerizing factor)
  VPFDAATLH
  VPFDAATLHT
  VPFDAATLHTSTA
  VPFDAATLHTSTAM
  VPFDAATLHTSTAMAAQHGMDDDGTGQK

PPD®

Multiplexed Immunoassay Panels

Migration Inhibitory Factor (MIF)

Leptin

Prolactin

Osteopontin

Cancer Antigen CA-125

IL-6

Prostate Specific Antigen (PSA)

Carcino-embryonic antigen (CEA)

Alpha fetoprotein (AFP)

Oncofetal Antigen CA-19-9

Cancer Antigen CA-15-3

Early Prostate Specific Antigen 1 or 2   (EPCA-1, -2)

Coagulation factor XIII

Antithrombin-III (ATIII)

Complement factor H-related protein 1 (FHR-1)

Extracellular matrix protein 1 (Secretory component p85)

Ficolin-3 (Collagen/fibrinogen domain-containing protein 3)

Gelsolin (Actin-depolymerizing factor / ADF)

Summary

Evaluate quantitatively major pre-analytical variables for plasma/serum collection

Perform LC-MS and Immunoassay protein measurements

Establish a standard panel of markers with which to grade blood protein integrity

Enable widespread use of this standard panel

Apply standard panel to evaluate existing collections