Multiplex measurement of tumor biomarkers associated with colorectal adenocarcinoma using novel sample types

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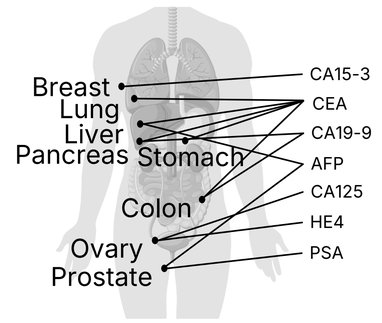
Summary

Discovery of new tumor biomarkers and their roles in cancer progression is an intense research area. Testing strategies that utilize a combination of proteins associated with cancers may improve diagnosis and monitoring chemotherapy in patients. Multiplex immunoassays are a valuable tool for investigating potential biomarkers in serum associated with cancer. We report here using a 28-plex Luminex performance immunoassay to measure tumor biomarkers in serum, plasma (EDTA, heparin, and Streck), and tissue lysates. Sera from late-stage colorectal cancer (CRC) patients had several potential tumor biomarkers at higher levels than sera from healthy donors. Tissue lysates prepared from late-stage CRC and healthy adjacent fresh-frozen tissue were used to measure CEA, CA19-9, IL-8, and MIF. The levels of MIF and IL-8 in these tissue lysates measured using Luminex was validated using the Jess capillary-based automated Western blot system. Stability testing of tumor biomarkers in EDTA, heparin, Streck® Cell-Free BCT®, and Streck Protein Plus plasma samples from healthy donors after 0, 3 and 5 days at room temperature revealed stabilization of most analyte concentrations using Streck tubes, with the exceptions of ENO-2 and CYFRA21-1. Multiplex immunoassays are a valuable tool for the discovery and development of soluble tumor biomarkers associated with cancer.

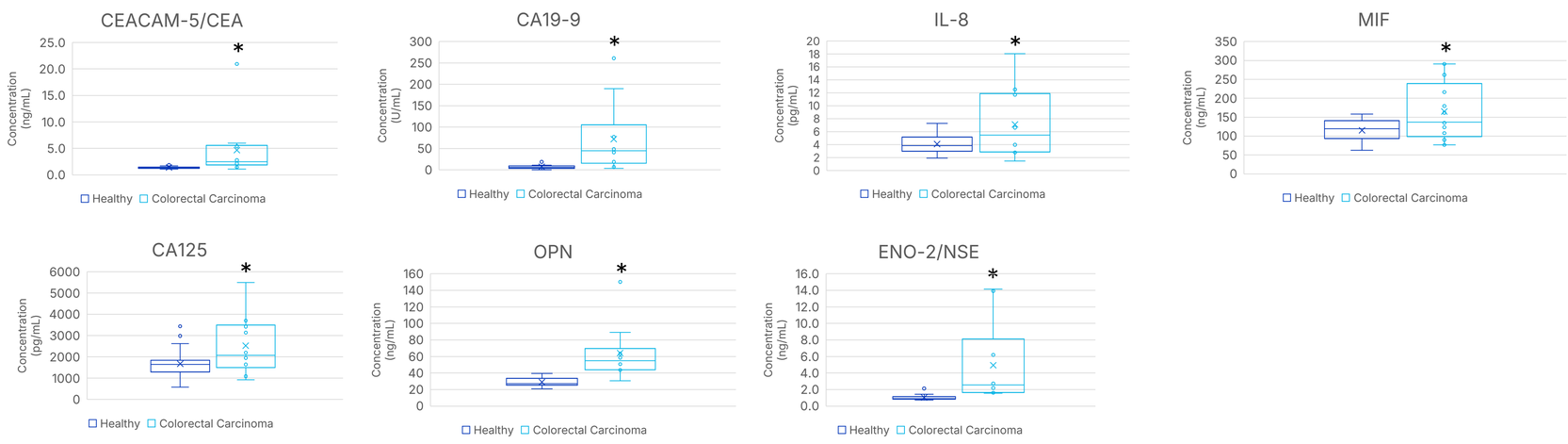
Luminex® Human Tumor Biomarker Panel, 28-plex

AFP/alpha Fetoprotein	CYFRA21-1	HE4/WFDC2	PSA, Total
CA125	ENO-2/NSE	HGF	Prolactin
CA15-3	Fas/TNFRSF6	IL-6	SCF
CA19-9	Fas Ligand/TNFSF6	IL-8/CXCL8	TGF alpha
CD40 Ligand	FGF basic	Leptin	Thyroglobulin
CEACAM-5/CEA	Glypican-1	MIF	TNF alpha
Chromogranin A	HCG-B	OPN	VEGF

Common Cancer Biomarkers



Tumor biomarkers in CRC sera



Analytes associated with CRC and additional analytes were observed at significantly higher levels in CRC than in healthy sera.
*Indicates p-value ≤ 0.05. Healthy (n=20) and CRC (n=10) sera were tested using the Human Tumor Biomarker Luminex Performance Assay.

Materials and Methods

Luminex Analysis

Sera, plasma, and tissue lysate samples were analyzed for 28 different analytes using the Human Tumor Biomarker Luminex Performance Panel (Bio-techne, Catalog # FCSTM25). Sera and Streck plasmas were diluted 2-fold and 8-fold with assay diluent. EDTA and heparin plasma were diluted 2-fold with assay diluent. Reported concentrations are back-calculated for dilution factor. Colon tissue lysates were run at 0.2 mg/mL (10 ug/well) and reported as pg (U for CA19-9) analyte/ug total protein.

Tissue Lysate Preparation

Fresh-frozen colon tissue from CRC and healthy adjacent tissue was verified by and obtained from Precision for Medicine (Frederick, MD). Tissue sections were weighed, dispersed by mechanical mincing, incubated on ice for 20 minutes in modified RIPA lysis buffer (Lysis Buffer 16, Bio-Techne, Catalog # 895935) containing 1X Protease Inhibitor Cocktail (Tocris, Catalog # 5500) and then sonicated 3-5 times with 20-30 sec bursts. After sonication, samples were clarified by centrifugation to remove suspended debris and lipids. Total protein concentration was determined via bicinchoninic acid assay (BCA).

Sample Sources and Stability Study

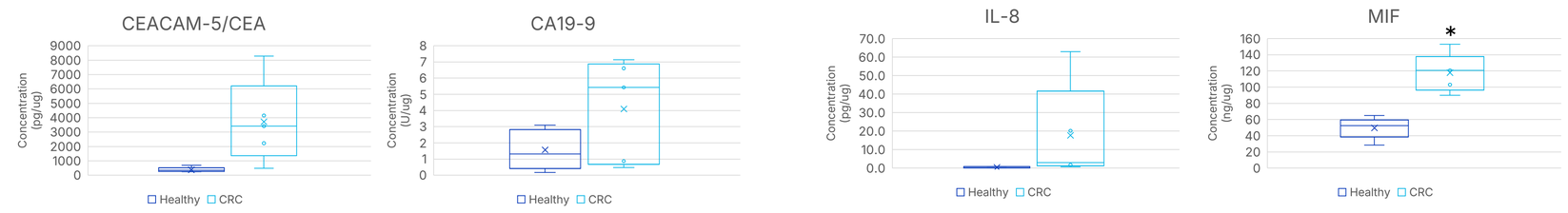
Colon cancer sera was purchased from BioIVT (Baltimore, MD). Additional serum was collected from healthy donors. Plasmas from healthy donors for the stability study were collected, incubated at room temperature for 0, 3, and 5 days before being processed and frozen. Streck Cell-free DNA BCT (Reference # 230470) and Streck Protein Plus (Reference # 230628) plasma collection tubes were obtained from Streck (La Vista, NE). Plasmas were tested using the Human Tumor Biomarker Luminex Performance Panel.

Immunoblot Analysis

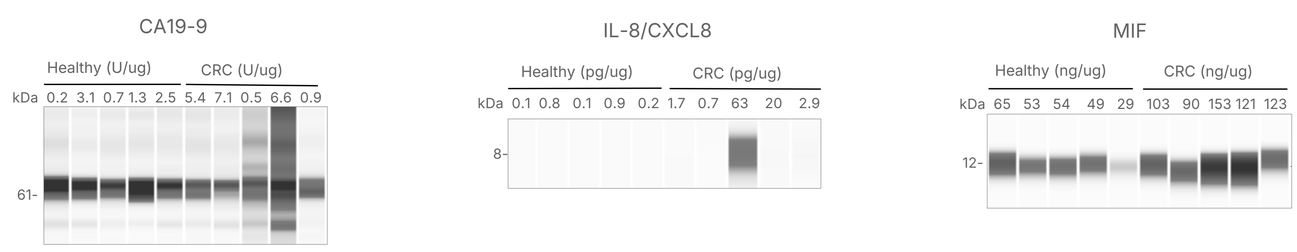
Tissue lysates were tested on the Simple Western capillary-based immunoassay instrument Jess (Protein Simple). MIF and IL-8/CXCL8 were tested using Goat Anti-Human/Mouse/Rat MIF Affinity Purified Polyclonal Antibody (Bio-Techne; Catalog # AF-289-PB) or Goat Anti-Human IL-8/CXCL8 Polyclonal Antibody (Bio-Techne; Catalog # AF-208-NA) followed by HRP-conjugated Anti-Goat Secondary Antibody (Bio-Techne; Catalog # HAF019). CA19-9 was tested on Jess using Mouse Anti-Human CA19-9 Monoclonal Antibody (Bio-Techne; Catalog # MAB10625) followed by HRP-conjugated Anti-Mouse Secondary Antibody (Bio-Techne; Catalog # HAF018). Specific bands were normalized to total protein measured using a Total Protein Detection Module (Part# DM-TP01).

Tumor biomarkers in CRC tissue lysates

Luminex

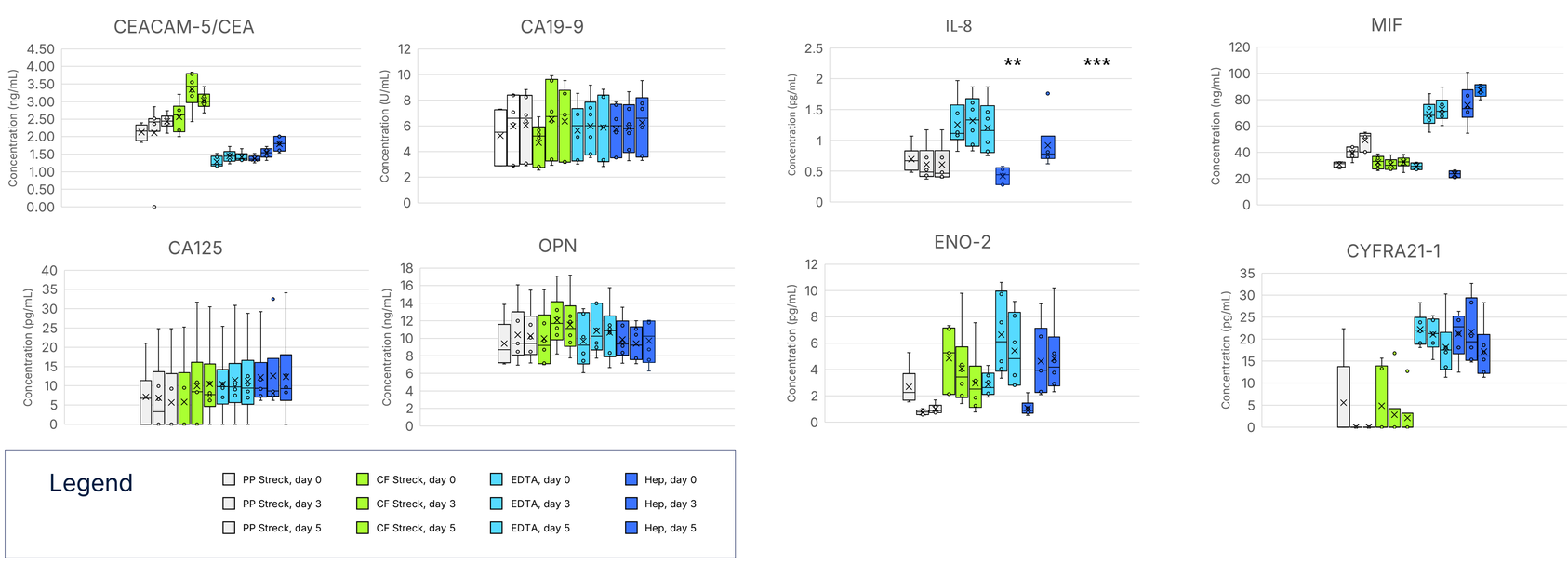


Jess (capillary-based immunoassay)



Analytes detected in CRC sera were detectable in CRC tissue lysates using the Human Tumor Biomarker Luminex Performance Assay. Jess capillary-based immunoassays show IL-8 and MIF at similar relative levels. CA19-9 was detected at 61 kDa using Jess and relative levels were not similar to Luminex. Observed concentrations in Luminex are shown above each lane in Jess data. *Indicates p-value ≤ 0.05.

Tumor biomarkers stability in Streck, EDTA, and heparin plasma samples



Streck Protein Plus and Cell-Free EDTA plasma showed better stability than EDTA and heparin plasma for most analytes. ENO-2/NSE and CYFRA21-1 in Protein Plus was less stable than other plasmas. Plasma collected from six donors were tested using the Human Tumor Biomarker Luminex Performance Assay. Release of MIF from platelets was diminished in Protein Plus and minimal in Cell-free Streck plasma, while detected at high levels on day 3 and day 5 in EDTA and heparin plasma. IL-8 is known to form heterodimers with a chemokine, PF4/CXCL4, released at high levels from platelets and is known to polymerize. **Day 3 and day 5 IL-8 concentrations in EDTA plasma ranged from 4.8 to 50 pg/mL. ***Day 3 and day 5 IL-8 concentrations in heparin plasma measured out of range high.

Conclusions

- The Human Tumor Biomarker Luminex Performance Assay (Cat # FCSTM25) measures biomarkers associated with colon cancer using serum, plasma, and tissue lysates.
- Jess capillary-based immunoassays can be used to validate Luminex assay results.
- Protein Plus and Cell-Free Streck plasma are novel sample types that are validated for most analytes in FCSTM25.
- Tissue lysates are a novel sample type for measuring tumor biomarkers using Luminex assays.
- The Human Tumor Biomarker Luminex Performance Assay enables the simultaneous quantitation of multiple tumor biomarkers in more sample types than any other Luminex assay.

References:

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