Utility of human and mouse multiplex immunoassay panels for studying profiles of circulating angiogenic factors as biomarkers for angiogenesis research

Wen-Rong Lie and Jehangir Mistry, Merck Millipore Corporation, 290 Concord Rd, Billerica, MA 01821, USA

Abstract

Monitoring the expression levels of multiple pro- and antiangiogenic factors in circulation is key in studying angiogenesis in normal physiology (such as wound healing) and pathological processes of diseases (such as cancer) or as biomarkers for angiogenesis therapy. Based on the Luminex xMAP® technology, we have recently developed two human angiogenesis panels and one mouse angiogenesis panel for the simultaneous detection of 37 and 27 angiogenic biomarkers in less than 50 µL of human or mouse samples, respectively. We report here secreted protein biomarker profiles using these multiple angiogenic factors /growth factors/ soluble receptors as well as the cytokines/chemokines in serum samples collected from cancer patients, pregnant individuals, and normal donors, and on cultured human and mouse tumor and stromal cells. The 2 human multiplex immunoassay panels are 1) MILLIPLEX® MAP Human Angiogenesis/Growth Factor Panel 1, a 17-plex multiplex immunoassay for the simultaneous quantification of 17 angiogenesis biomarkers: Angiopoietin-2, BMP-9, EGF, Endoglin, Endothelin-1, FGF-1, FGF-2, Follistatin, G-CSF, HB-EGF, HGF, IL-8, Leptin, PLGF, VEGF-A, VEGF-C and VEGF-D, and 2) MILLIPLEX® MAP Human Angiogenesis Panel 2, a 20-plex multiplex immunoassay for simultaneous quantitation of 20 human angiogenesis biomarkers: Angiostatin, sE-Selectin, Osteopontin, PDGF-AB/BB, sCD31/PECAM-1, sNeuropilin-1, sVEGFR 1, sVEGFR2, and sVEGFR3, sTie-2, Tenascin C, Thrombospondin-2 (TSP-2), sAXL, sc-Kit, sEGFR, sHer2/ErbB2, sHer3/ErbB3, sHGFR/c-Met, sIL-6Rα, soluble urokinase plasminogen activator receptor (suPAR). The mouse multiplex immunoassay panel is MILLIPLEX® MAP Mouse Angiogenesis/Growth Factor Panel, a 27-plex, for the simultaneous quantitation of 27 mouse angiogenesis biomarkers: Amphiregulin, Angiopoietin-2, Betacellulin, sCD31/PECAM-1, EGF, Endoglin, Endothelin-1, sFasL, FGF-1, Follistatin, G-CSF, HGF, IL-1β, IL-6, IL-17A, KC/CXCL1, Leptin, MCP-1/CCL2, MIP-1α/CCL3, PLGF-2, Prolactin, SDF-1/CXCL12, TNFα, VEGF-A, VEGF-C, and VEGF-D. The results of this study demonstrated the utility of these human and mouse angiogenesis panels and cytokine/chemokine panels in studying secretome profiles related to angiogenesis, as well as the potential application in cancer research and angiogenesis therapy.

Methods

Serum and Culture Supernatant Samples: Human serum samples were purchased from Discovery Life Sciences, Inc. and BioreclamationIVT, including colorectal 20 cancer serum samples, 20 cancer serum samples, 20 normal serum samples, and human serum samples in pregnancy. All cell lines listed were obtained from ATCC®.

Multiplex Protein Biomarker Immunoassays: All human serum samples were tested for 37 circulating angiogenesis biomarkers using two MILLIPLEX® MAP Immunoassay Panels (Merck Millipore): Human Angiogenesis/Growth Factor Panel 1 (HAGP1MAG-12K, 17-plex) for 1:3 diluted serum samples, and Human Angiogenesis Panel 2 (HAGP2MAG-12K, 20-plex) for 1:5 diluted serum samples. Human cell culture supernatants were evaluated as neat or diluted samples for the same 2 human angiogenesis panels; mouse cell culture supernatants were tested using a Mouse Angiogenesis/Growth Factor Panel (MAGPMAG-24K, 27-plex). Samples were analyzed with the MILLIPLEX® panels on a Luminex 200™ System with MILLIPLEX® Analyst 5.1 and xPONENT® software.

Human Angiogenesis Panel Assay Method: The MILLIPLEX® assay was performed in a 96-well plate. The protocol is as follows: wet the plate with 200 µL assay buffer → 25 µL matrix/assay buffer, 25 µL standards/samples, 25 µL capture antibody conjugated magnetic beads → incubate at 4ºC, overnight (or at RT, 2 hr) → wash plate → add 25 µL biotinylated detection Abs, RT, 1 hr → add 25 µL Streptavidin-Phycoerythrin (SAPE), RT, 30 min → wash plate → add 100 µL sheath fluid → read on a Luminex instrument.

Corresponding Author: wen-rong.lie@merckmillipore.com

Results

Figure 1. Standard Curves for Human or Mouse Angiogenesis Panels

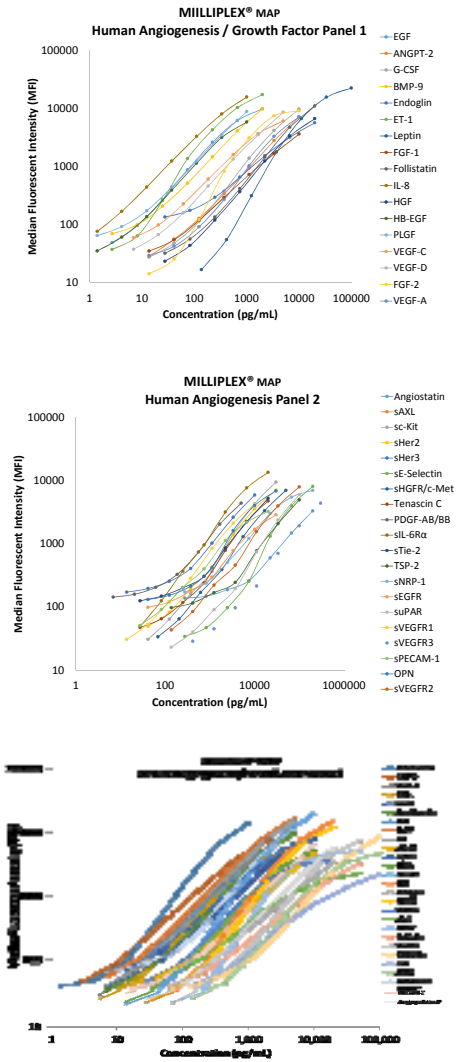
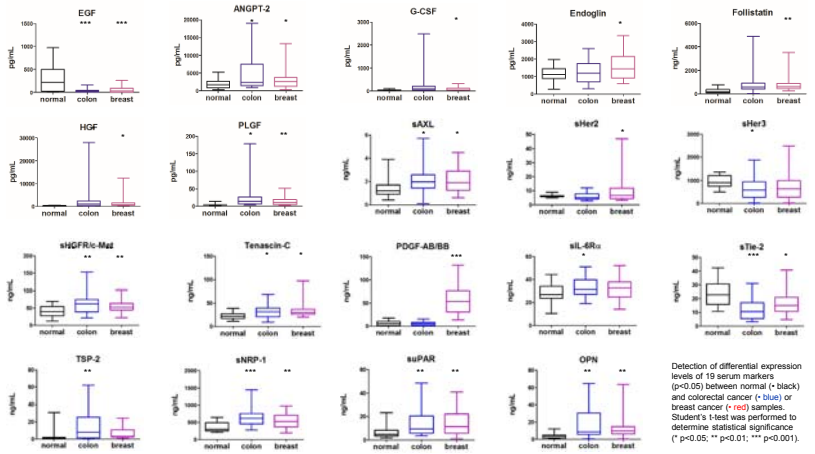
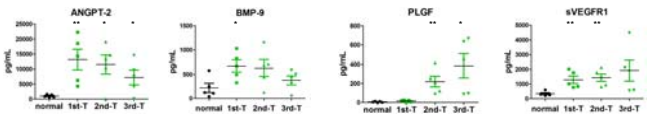


Figure 2. Quantification of Angiogenesis Protein Biomarkers in Human Colorectal and Breast Cancer Serum Samples



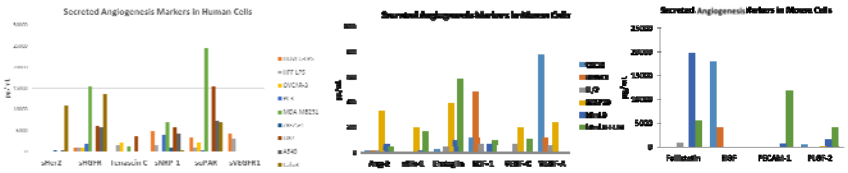
Detection of differential expression levels of 19 serum markers ($p<0.05$) between normal (• black) and colorectal cancer (• blue) or breast cancer (• red) samples. Student's t-test was performed to determine statistical significance (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Figure 3. Quantification of Circulating Angiogenesis Protein Biomarkers in Serum of Human Pregnant Subjects



Detection of differential expression levels of 5 human pregnancy serum markers ($p<0.05$) between "normal" (• black) and each of the 3 trimester stages in pregnancy (• green) samples. Student's t-test was performed for statistical significance (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Figure 4. Quantitative Profiling of Secreted Angiogenesis Markers in Human & Mouse Cell Culture Conditioned Media



Summary

- We developed two human multiplex immunoassay panels (Human Angiogenesis Panel 1 & Panel 2) for quantification of 37 angiogenesis biomarkers in less than 50 µL of human serum samples.
- Preliminary analysis shows differential expression of multiple circulating biomarkers in "normal" vs. breast or colon cancer and in "normal" vs. pregnancy in human serum samples.
- We also developed a Mouse Angiogenesis Panel for simultaneously quantifying 27 angiogenesis / growth factor biomarkers for mouse research and preclinical studies.
- MILLIPLEX® MAP Human and Mouse Angiogenesis Panels are ideal solutions for translational researchers studying angiogenesis biomarkers in normal vascular development and pathogenesis of tumor angiogenesis.