Associated multiplex biomarker detection for colorectal cancer based on Bio-Plex platform

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Background: Associated protein biomarkers are thought to be produced by the tumor or other tissues in response to the existence of cancers or associated conditions. Equally, known examples of cancer protein biomarker are used for the diagnosis of colorectal cancers (CRCs). However, these biomarkers suffer from low diagnostic specificity and sensitivity for CRC patients. Single biomarker detection is rarely useful for clinical applications due to the heterogeneity of cancer. Therefore, new sensitive and specific assays are urgently required for CRC diagnosis at an early stage.

Methods: In this study, a total of 58 newly diagnosed primary CRC patients in Dianjiang People's Hospital of Chongqing were included, while 20 controls were also included. Approximately 2 mL of venous blood samples from 58 CRC patients and 20 controls were collected. Then we detected 16 angiogenic cytokines using the Bio-Plex technology to measure multiple cytokines.

Results: The results showed that serum levels of Follistatin, HGF, and Osteopontin were significantly higher in CRC patients, while Leptin and PECAM were significantly decreased. ROC curve analyses indicated that the up-regulated markers, including Follistatin, HGF, and Osteopontin, had diagnostic value for CRC. The down-regulated marker, PECAM, also presented diagnostic value for CRC. To maximize the ability to detect CRC, four biomarkers, as well as CEA, were combined and used for linear discriminant analysis. Using the regression equation, the sensitivity and specificity for CRC were calculated as 93.1% (54/58) and 60.0% (12/20).

Conclusions: The study demonstrates that the combined analysis of five cytokines (follistatin, HGF, leptin, PECAM and osteopontin) is more useful for CRC diagnosis than the analysis of any individual marker. The combined detection of these five biomarkers is a valuable method for the diagnosis of CRC.

Keywords: Multiplex immunoassay; plasma biomarker; colorectal cancer (CRC)

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Introduction

Colorectal cancer (CRC) is the fifth leading cause of cancer-related death in China. It is estimated that about 2,814,000 Chinese die from cancer in 2015, corresponding to over 7,500 cancer deaths on average per day, and a total of 215,700 new CRC cases and 376,300 deaths were estimated to occur in 2015 (1). Despite the advances in CRC therapy, the survival rate of CRC patients remains low because most CRC patients are diagnosed at the advanced stage. There are several ways to diagnose CRC, such as colonoscopy, CT scan, tumor biomarkers, stool occult blood test and so on. However, a convenient way for CRC screening is currently lacking. Thus, new convenient, sensitive and specific diagnostic methods for CRC are urgently required. CRC research has focused mainly on intracellular biomarkers for

Table 1 The clinical features of the 58 colorectal cancer patients

Features	Values
Age (years)	66.5 (38.1 to 86.5)
Gender	
Male	37 (63.8%)
Female	21 (36.2%)
Location	
Left hemicolon	35 (60.3%)
Right hemicolon	23 (39.7%)
TNM stage	
T1–T2	11 (19.0%)
T3–T4	47 (81.0%)
Metastasis	
With	27 (46.6%)
Without	31 (53.4%)
Differentiation	
Well	7 (12.1%)
Moderately	19 (32.8%)
Poorly	32 (55.2%)

many years (2). Recently, more attention has been paid to soluble cancer biomarkers, which can be used for the earlier diagnosis of CRC (3).

A cancer biomarker refers to a substance or process that is indicative of the presence of cancer in the body. The National Institutes of Health Biomarkers Definition Working Group1 provided a formalized definition of biomarker as cellular, biochemical, and molecular alterations by which a normal, abnormal, or simply biologic process can be recognized or monitored and are used to objectively measure and evaluate normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (4). Most diagnostic tests available to date are based on a single protein biomarker. So far, some serum tumor markers are used for the adjunctive diagnosis of CRC. But each single biomarker suffers from low diagnostic specificity and sensitivity because of the heterogeneity of cancer phenotypes (5). It is now widely accepted that panels of biomarkers are required to achieve the increased sensitivity and specificity necessary for population-based screening (6). Therefore, we try to use the detection of associated multi-parametric tumor biomarkers for clinical CRC diagnosis.

The Bio-Plex technology that combines fluorescent flow cytometry and ELISA is an accurate and sensitive method for detecting multiple cytokines (7). It also can be used to quantify multiple protein biomarkers in a single well of a 96-well plate simultaneously. Based on Bio-Plex technology, human cancer biomarker assays are designed to meet the needs of the most demanding clinical research environments. We measured the differences between CRC patients and healthy volunteers in 16 angiogenic molecules in serum samples. We also tried to analyze the clinical significance of these biomarkers in CRC patients. Furthermore, we evaluated the diagnostic values of each single factor and the combination of multiple biomarkers for CRC.

Methods

Samples

A total of 152 patients were identified from participants from Dianjiang area and for whom stored plasma samples were available. Samples were selected as follow: (I) patients were newly diagnosed primary CRC in Dianjiang People's Hospital of Chongqing; (II) patients who had received any anti-cancer therapy were excluded from the study; (III) patients who suffered from any other cancer (such as breast cancer, lung cancer, etc.) were excluded from the study. In the end, a total of 58 newly diagnosed primary CRC patients in Dianjiang People's Hospital of Chongqing were included in the study. Twenty controls were also included in the study, to compare CRC patients' biomarker level with those in the normal population. No difference in age and gender was observed between controls and cases. Each participant provided written informed consent, and the study was authorized by the Ethics Committee of Dianjiang People's Hospital (ID: CQDY2015071421).

The clinical parameters of 58 patients, including age, gender, location, T stage and LN metastasis, were collected from the patient records and summarized in *Table 1*. The histopathological diagnosis of the specimens was made according to the AJCC TNM classification (7th edition). Subjects in the healthy control group (n=20) ranged in age from 35.6 to 84.9 years.

Serum collection

Approximately 2 mL of venous blood sample from 58 CRC patients and 20 controls were collected. Then these samples were drawn into tubes without anticoagulant agent and then

centrifuged. All serum samples were frozen at –80 ℃ until used.

Luminex analysis

A Bio-Plex suspension array system (Human Cancer Biomarker 16-Plex Panel, Bio-Rad) was performed to detect the levels of 16 types of serum cytokines. The Bio-Plex Human Cancer Cytokine 16-Plex panel includes human sEGFR, FGF-basic, Follistatin, G-CSF, sHER2/neu, HGF, sIL-6Ra, Leptin, Osteopontin, PDGF-AB/BB, Hu PECAM, Prolactin, SCF, sTIE-2, sVEGFR-1, sVEGFR-2. Premixed beads coated with the 16 types of target capture antibodies were transferred into a 96-well filtration plate supplied with the assay kit. After two washes with the Bio-Plex wash buffer, premixed standards or 50 µL samples were added to each well containing washed beads. Then, the 96-well filtration plate was placed on a shaking table (850±50 rpm) for 1 hour at room temperature, to permit each bead to adequately bind to the specific biomarker. After two washes with 100 µL Bio-Plex wash buffer, mixed biotinylated detection antibodies (50 µL, final concentration of 2 µg/mL) were added to each well and incubated for 10 minutes at room temperature on the shaking table. After three washes with 100 µL Bio-Plex wash buffer, the beads were re-suspended in 125 µL of Bio-Plex assay buffer and read on a Bio-Plex array reader. The data were analyzed using BioPlex® 2000 Multiplexing Platform.

Data analysis

All statistical analyses were performed using the SPSS 17.0 software package (SPSS, Chicago, IL), and values of $P<0.05$ were defined as statistically significant. The data are expressed as the means ± standard error of measurements. We get the original data from the BioPlex® 2000 Multiplexing Platform. All the data in the text are from Table S1. First, the Wilcoxon rank sum test was used to analyze the differential expression of factors between controls and CRC patients. Then we screened out some cytokines that are valuable in the diagnosis of CRC. The Kruskal-Wallis test was performed to evaluate whether the levels of various factors were correlated with clinical parameters. The diagnostic value of each marker was determined by receiver operating characteristic (ROC) curve analysis. At last, we use linear discriminant analysis (stepwise method) to verify associated markers of CRC diagnosis.

Results

The characteristics of CRC patients

The clinical features of the 58 patients who were diagnosed CRC are presented in *Table 1*. The patients included 37 (63.8%) men and 21 (36.2%) women. The average age is 66.5 years (66.5±10.2, from 38.1 to 86.5). Among these patients, well differentiated cancers were diagnosed in 7 (12.1%) patients, moderately differentiated cancers in 19 (32.8%) patients and poorly differentiated cancers in 32 (55.2%) patients. CT scan showed that 27 (46.6%) patients had lymph node metastases, while other 31 (53.4%) patients not.

Profiles

The expression levels of 16 cytokines in each of the plasma samples from CRC patients and healthy volunteers were evaluated by using Bio-Plex assay. Then we use Wilcoxon rank sum test to analyze the differential expression of factors between controls and CRC patients. The levels of 3 cytokines, follistatin (997.4±87.2 *vs.* 586.7±40.8 pg/mL), HGF (2,076.2±141.2 *vs.* 1,430.7±54.7 pg/mL), osteopontin (87,317.3±7,027.8 *vs.* 57,286.9±5,192.1 pg/mL), PECAM (10,016.2±382.5 *vs.* 7,863.5±499.8 pg/mL), are significantly higher in CRC patients compared with healthy controls (*Table 2*). We also detected a significant decrease of leptin (4,483.6±763.7 *vs.* 9,530.6±1,760.3 pg/mL), in CRC patients compared with healthy controls (*Table 2*). There is no significant difference in sEGFR, FGF-basic, G-CSF, sHER2/neu, sIL-6Ra, PDGF-AB/BB, Prolactin, SCF, sTIE-2 sVEGFR-1, or sVEGFR-2 levels was observed between cancer patients and healthy volunteers ($P>0.05$).

Correlation of cytokine levels and clinical parameters in CRC patients

We further analyzed whether the levels of these cytokines were correlated with clinical parameters in CRC patients. Mann-Whitney U analyses showed that leptin ($P=0.026$) level was significantly higher in patients with advanced CRC. The levels of leptin ($P=0.017$) and PECAM ($P=0.016$) were significantly correlated with the cancer differentiation state. Moreover, plasma levels of osteopontin ($P=0.042$) were significantly higher in patients with lymph node metastasis than in patients without lymph node metastasis (*Table 3*). We did not find any association between the levels of other factors and clinical parameters (*Table 3*).

Table 2 Correlation of 5 angiogenic cytokines in patients with colorectal cancer

Cytokine	Colorectal cancer (pg/mL)	Control (pg/mL)	P value
Follistatin	997.4±87.2	586.7±40.8	0.008
Osteopontin	87,317.3±7,027.8	57,286.9±5,192.1	0.018
PECAM	10,016.2±382.5	7,863.5±499.8	0.04
HGF	2,076.2±141.2	1,430.7±54.7	0.01
Leptin	4,483.6±763.7	9,530.6±1,760.3	0.03

Table 3 The Asymp. Sig of 5 cytokines correlated with clinical parameters

Cytokine	Differentiation state	TNM stages	LN metastasis
Follistatin	0.159	0.498	0.876
HGF	0.822	0.926	0.398
Leptin	0.017	0.026	0.809
Osteopontin	0.786	0.864	0.042
PECAM	0.016	0.641	0.537

Table 4 The cutoff thresholds for each biomarker

Cytokine	AUC	Standard error	z statistic	P value
Follistatin	0.695	0.0589	3.315	0.0009
HGF	0.852	0.0425	8.286	<0.0001
Leptin	0.728	0.0655	3.488	0.0005
Osteopontin	0.671	0.0642	2.658	0.0079
PECAM	0.734	0.0646	3.632	0.0003

AUC, area under the ROC curve.

Screening and diagnostic serum biomarkers for CRC

ROC curve analyses were performed to evaluate the diagnostic values of individual marker for CRC. Among the 16 biomarkers tested, the up-regulation of follistatin (0.695, P=0.0009), HGF (0.852; P<0.0001) and osteopontin (0.671; P=0.0079) were shown to have significant diagnostic values for CRC. Among the decreased markers, only PECAM (0.734; P=0.0003) exhibited significant diagnostic values for CRC (*Table 4*). These cutoff values were calculated according to the principle that the maximum sensitivity values added specificity (*Table 5*, *Figure 1*).

Associated markers for CRC diagnosis

To maximize the ability to detect CRC, the concentrations of serum follistatin, osteopontin, PECAM, HGF and leptin were further used for linear discriminant analyses. Using a stepwise method, we obtained the following regression equations: Y=0.03 Follistatin +0.04× HGF. Using this regression equation, the sensitivity and specificity for CRC were calculated as 93.1% (54/58) and 60.0% (12/20), indicating that the combination of multiple markers is a more powerful tool for CRC diagnosis than each individual marker detection.

Discussion

CRC is a leading cause of death worldwide, with over one million of new cases and half a million of deaths around the world every year. Approximately 1,235,108 people

Table 5 The sensitivity and specificity for each cutoff threshold

Cytokine	Youden index	Threshold	Sensitivity (%)	Specificity (%)
Follistatin	0.4190	>721.455	56.90	85.00
HGF	0.6741	>1,712.22	72.41	95.00
Leptin	0.4034	≤2,467.32	60.34	80.00
Osteopontin	0.3345	>69,688.73	53.45	80.00
PECAM	0.4379	>9,155.08	63.79	80.00

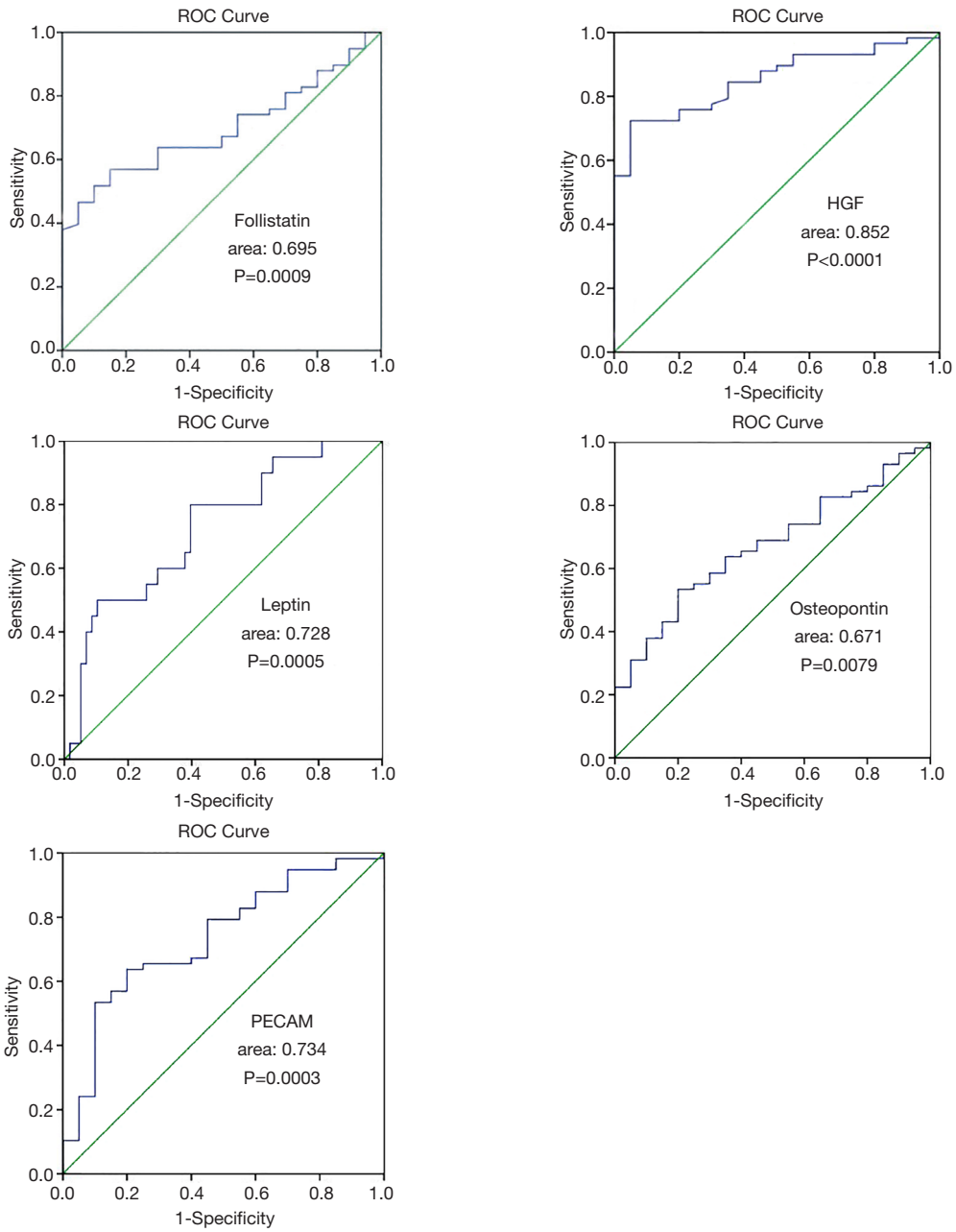

Figure 1 The AUC for each biomarker. AUC, area under the ROC curve.

are diagnosed annually with CRC, from which 609,051 die annually (8) .The World Health Organization estimates an increase of 77% in the number of newly diagnosed cases of CRC and an increase of 80% in deaths from CRC by 2030 (9). Five-year relative survival rates are under 50%, this greatly depends on the stage at the time of diagnosis (10). No primary preventive measure has proven efficacy in reducing incidence, but early detection through population screening has been found to reduce mortality (11). Unfortunately, many patients have distant metastatic disease at the time of presentation (12). Therefore, it's necessary for early diagnosis of CRC. There are many methods for the diagnosis of CRC, including electronic colonoscopy, CT scan, serum tumor markers, and so on. Colonoscopy, which has higher sensitivity (97%) and specificity (98%) for early detection of CRC, is also invasive, expensive, and has a high risk of complications, which often leads to poor patient compliance (13,14). The traditional detection of serum tumor markers is more convenient, but lack of specificity and sensitivity. So we want to find a new way to solve this problem.

Recently, the Luminex platform has become a robust, mainstream approach for academic and pharmaceutical research. This technology gives researchers the ability to look at analyse simultaneously providing more information from less sample volume in less time than traditional immunoassay methods (15). Abnormal plasma levels of cytokines were proved to be associated with different clinical manifestations in patients by using this technology (16).

Some experiments prove that serum follistatin is aberrant expressed in a variety of solid tumours, including breast cancer (17), prostate cancer (18), lung cancer (19), and melanoma (20). Abnormal activation of HGF is also established in different kinds of cancer, including myeloma, acute myeloid leukemia, chronic myelogenous leukemia, and myeloproliferative neoplasms (21). It was observed that more colon tumors were found in mice which fed a high-fat diet (22). It was proved that serum leptin levels were significantly decreased in patients with colon cancer (23). Many researchers believe that plasma osteopontin is a potential diagnostic biomarker for hepatocellular carcinoma (24), gastric cancer (25), melanoma (26), lung cancer (27), prostate cancer (28), and ovarian cancer (29,30). PECAM is thought to be associated with breast cancer (31). In our study, we found the five biomarkers, follistatin, HGF, osteopontin, PECAM and leptin were significantly changed in CRC patients. Therefore, examinations of these five serum cytokines at the same time maybe can improve the diagnostic rate of patients with CRC.

In the present study, we describe the characterization of a novel blood test for CRC patients for the first time based on the novel, high-flux Bio-Plex assay platform. The results showed that serum levels of Follistatin, HGF, PECAM, and osteopontin were significantly higher in CRC patients than healthy subjects, while serum levels of leptin were decreased. The levels of and leptin and PECAM were significantly correlated with tumor differentiation status. Moreover, serum levels of osteopontin were significantly higher in patients with lymph node metastasis compared with patients without lymph node metastasis. But the limitation of our study is that the mechanism of these serum biomarkers for CRC is not clear yet. This is what we need to do in our next study.

ROC curve analyses indicated that markers that were up-regulated in CRC, including follistatin, HGF, and osteopontin, had good diagnostic value. The down-regulated marker, PECAM, also presented a good diagnostic value for CRC. However, due to the heterogeneity of the tumors, individual marker detection would be insufficient for clinical evaluation. Therefore, multiplex marker detection provides numerous advantages as a diagnostic platform. To maximize the ability to detect CRC, the five identified biomarkers were further used for linear discriminant analyses. Using a regression equation, the sensitivity and specificity for CRC were found to be 93.1% (54/58) and 60.0% (12/20), respectively, indicating that the multiplex method is a powerful tool for CRC diagnosis compared with single marker detection.

In conclusion, the present study examined the differential expression and diagnostic values of 16 angiogenic molecules using a Bio-Plex bead-based liquid suspension array. This platform offers ease of use, low cost, flexible array preparation, and automatic data analysis, making it an attractive platform for widespread applications. Further study will be required to validate the practicality of the regression equation and to identify the best combination of multiplex markers for CRC diagnosis based on the Bio-Plex platform.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/amj.2019.03.01). The authors have no conflicts of interest declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Each participant provided written informed consent, and the study was authorized by the Ethics Committee of Dianjiang People's Hospital (ID: CQDY2015071421).

References

1. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016 ;66:115-32.
2. Vacante M, Borzì AM, Basile F, et al. Biomarkers in colorectal cancer: Current clinical utility and future perspectives. World J Clin Cases 2018;6:869-81.
3. Mohammadi P, Saidijam M, Kaki A, et al. Pilot Study of CK19, CK20 and GCC mRNA in the Peripheral Blood as a Colorectal Cancer Biomarker Panel. Int J Mol Cell Med 2016;5:30-36.
4. Wagner PD, Srivastava S. New Paradigms in Translational Science. Research in Cancer Biomarkers. Transl Res 2012;159:343-53.
5. Mahboob S, Ahn SB, Cheruku HR, et al. A novel multiplexed immunoassay identifies CEA, IL-8 and prolactin as prospective markers for Dukes' stages A-D colorectal cancers. Clin Proteomics 2015;12:10.
6. Nice E. Biomarker discovery and validation: the tide is turning. Expert Rev Proteomics 2013;10:505-7.
7. Ribeiro S, Nascimento H, Borges A, et al. Comparison of Bio-Plex measurements with standard techniques. Clin Chem Lab Med 2011;50:399-402.
8. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 2010;46:765-81.
9. Binefa G, Rodríguez-Moranta F, Teule A, et al. Colorectal cancer: From prevention to personalized medicine. World J Gastroenterol 2014;20:6786-808.
10. Kim HJ, Yu MH, Kim H, et al. Noninvasive molecular biomarkers for the detection of colorectal cancer. BMB Rep 2008;41:685-92.
11. Burt RW. Colorectal cancer screening. Curr Opin Gastroenterol 2010;26:466-70.
12. Sag AA, Selcukbiricik F, Mandel NM. Evidence-based medical oncology and interventional radiology paradigms for liver-dominant colorectal cancer metastases. World J Gastroenterol 2016;22:3127-49.
13. Regula J, Rupinski M, Kraszewska E, et al. Colonoscopy in colorectal-cancer screening for detection of advanced neoplasia. N Engl J Med 2006;355:1863-72.
14. Quintero E, Castells A, Bujanda L, et al. Colonoscopy versus fecal immunochemical testing in colorectal-cancer screening. N Engl J Med 2012;366:697-706.
15. Houser B. Bio-Rad's Bio-Plex® suspension array system, xMAP technology overview. Arch Physiol Biochem 2012;118:192-6.
16. Day JB, Basavanna U. Magnetic bead based immuno-detection of Listeria monocytogenes and Listeria ivanovii from infant formula and leafy green vegetables using the Bio-Plex suspension array system. Food Microbiol 2015;46:564-72.
17. Zabkiewicz C. Increased Expression of Follistatin in Breast Cancer Reduces Invasiveness and Clinically Correlates with Better Survival. Cancer Genomics Proteomics 2017;14:241-51.
18. Su S. Up-Regulation of Follistatin-Like 1 By the Androgen Receptor and Melanoma Antigen-A11 in Prostate Cancer. Prostate 2017;77:505-16.
19. Bae K. Mitotic cell death caused by follistatin-like 1 inhibition is associated with up-regulated Bim by

inactivated Erk1/2 in human lung cancer cells. Oncotarget 2016;7:18076-84.
20. Shi L, Resaul J, Owen S, et al. Clinical and Therapeutic Implications of Follistatin in Solid Tumours. Cancer Genomics Proteomics 2016;13:425-35.
21. Boissinot M, Vilaine M, Hermouet S. The Hepatocyte Growth Factor (HGF)/Met Axis: A Neglected Target in the Treatment of Chronic Myeloproliferative Neoplasms? Cancers (Basel) 2014;6:1631-69.
22. Sung MK, Yeon JY, Park SY, et al. Obesity-induced metabolic stresses in breast and colon cancer. Ann N Y Acad Sci 2011;1229:61-8.
23. Yehuda-Shnaidman E, Nimri L, Tarnovscki T, et al. Secreted Human Adipose Leptin Decreases Mitochondrial Respiration in HCT116 Colon Cancer Cells. PLoS One 2013;8:e74843.
24. Tsuchiya N, Sawada Y, Endo I, et al. Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol 2015;21:10573-83.
25. Cao DX, Li ZJ, Jiang XO, et al. Osteopontin as potential biomarker and therapeutic target in gastric and liver cancers. World J Gastroenterol 2012;18:3923-30.
26. Filia A, Elliott F, Wind T, et al. Plasma osteopontin concentrations in patients with cutaneous melanoma. Oncol Rep 2013;30:1575-80.
27. Rud AK, Lund-Iversen M, Berge G, et al. Expression of S100A4, ephrin-A1 and osteopontin in non-small cell lung cancer. BMC Cancer 2012;12:333.
28. Thoms JW, Dal Pra A, Anborgh PH, et al. Plasma osteopontin as a biomarker of prostate cancer aggression: relationship to risk category and treatment response. Br J Cancer 2012;107:840-46.
29. Jahns F, Wilhelm A, Jablonowski N, et al. Butyrate suppresses mRNA increase of osteopontin and cyclooxygenase-2 in human colon tumor tissue. Carcinogenesis 2011;32:913-20.
30. Takami Y, Russell MB, Gao C, et al. Sp1 Regulates Osteopontin Expression in SW480 Human Colon Adenocarcinoma Cells. Surgery 2007;142:163-69.
31. Wang Z, Lei L, Cai XJ, et al. A preliminary study of pamidronic acid downregulation of angiogenic factors IGF-1/PECAM-1 expression in circulating level in bone metastatic breast cancer patients. Onco Targets Ther 2016;9:3147-52.

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Supplementary

Table S1 2017-6-30 multiple detection results

Type	Well	Group	Obs Conc														
			Hu sEGFR (15%)	Hu FGF-basic (44%)	Hu Follistatin (26%)	Hu G-CSF (57%)	Hu sHER2/neu (12%)	Hu HGF (62%)	Hu sIL-6Ra (19%)	Hu leptin (78%)	Hu osteopontin (77%)	Hu PDGF-AB/BB (47%)	Hu PECAM (46%)	Hu prolactin (52%)	Hu SCF (65%)	Hu sTIE-2 (64%)	Hu sVEGFR-1 (76%)	Hu sVEGFR-2 (45%)
B	G3, H3																	
S1	A1, A2	Standard sample		14,911.63	28,955.59	23,607.48	25,272.74	36,528.58	14,122.73	166,261.89	208,176.74	24,402.39	159,143.75	222,670.83	25,222.17	190,089.49	53,543.95	201,793.05
S2	B1, B2	Standard sample	55,081	5,408.24	7,037.81	5,694.1	6,692.46	9,038.1	3,473.75	31,211.92	60,669.63		39,150.6	53,996.78	6,470.22	47,493.96	12,936.85	51,455.93
S3	C1, C2	Standard sample	13,766.52	1,104.47	1,818.26	1487.74	1,584.51	2,324.27	897.56	8,863.76	13,664.65	1,537.89	9,931.68	13,830.09	1,575.43	11,889.34	3,490.35	12,623.08
S4	D1, D2	Standard sample	3,444.98	304.74	452.84	351.27	396.51	550.29	215.77	2,145.27	35,67.89	376.95	2,457.65	3,503.12	398.3	2,959.05	780.98	3,160.44
S5	E1, E2	Standard sample	857.88	70.39	107.94	91.77	106.72	149.76	55.6	533.03	876.54	97.94	618.23	827.64	101.73	751.62	227	812.44
S6	F1, F2	Standard sample	217.65	19.09	29.63	23.02	24.48	33.57	13.56	135.91		23.24			23.99	178.9	41.15	193.67
S7	G1, G2	Standard sample	51.82	3.89		5.75	6.11	9.19	3.59			6.19		59.61	6.56	52.71	33.1	50.71
S8	H1, H2	Standard sample		0.89	1.26	1.09	1.71	2.52	0.77	7.54	15.18	1.43	9.03	11.07	1.48	8.63		12.28
X1	A12	6	44,211.47	213.57	658.82	151.72	8522.48	1,717.68	48,887.87	23,707.26	76,556.7	1,392.24	91,42.31	17043	369.23	10,424.72	392.19	3,289.23
X2	B12	7	45,478.71	230.75	602.6	163.64	11967.84	2,182.23	37,163.24	24,818.91	133,713.22	827.13	81,33.29	15,981.53	364.12	12,068.63	286.34	3,101.82
X3	C12	8	46,663.76	251.3	887.8	210.92	9073.36	1,919.3	47,745.99	1,339.16	68,659.82	1,445.22	7,093.61	14,018.02	189.84	9,230.19	423.28	3,057.46
X4	D12	9	28,770.48	248.14	481.52	107.08	7695.17	1,941.04	30,495.64	1,080.61	85,731.15	881.91	12,052.13	16,619.18	168.57	9,126.82	374.47	2,137.4
X5	E12	11	46,420.35	187.73	658.82	139.61	8152.09	1,772.28	33,660.22	3,860.57	88,924.53	567.91	6,145.14	13,211.89	154.81	12,434.07	303.9	3,180.71
X6	F12	12	49,425.52	231.87	504.12	180.05	11709.68	2,363.17	40,298.81	1,813.67	66,974.2	934.49	19,823.45	301,276.49	219.47	16,380	512.57	2,865.36
X7	G12	14	33,966.77	275.7	1190.99	224.36	5354.61	2,471	25,303.08	2,221.52	62,842.79	2,094.17	7,309.92	4,045.19	222.76	8,631.65	454.45	2,530.76
X8	H12	15	37,926.69	196.61	548.89	169.54	12040.74	1,575.29	38,697.75	4,952.08	79,433.8	941.15	8,472.55	68,542.91	304.99	14,792.31	286.34	1,640.41
X9	A11	17	37,852.88	219.41	1077.31	191.6	6331.18	1,913.87	49518.5	2,287.39	167,362.29	366.06	12,558.45	22,433.43	173.78	17,902.16	321.49	3,872.69
X10	B11	18	29,627.36	194.1	516.96	124.8	8795.56	1,514.83	33,103.82	2,398.7	146,763.08	500.03	9,789.64	27,195.73	218.54	11,925.93	225.2	2,752.14
X11	C11	21	33,662.8	197.86	382.76	137.17	8523.27	2,230.88	27,949.5	2,430.81	83,420.89	1,142.79	9,218.92	16,203.05	378.07	11,346.36	233.9	2,584.86
X12	D11	26	41,119.54	202.78	362.55	161.27	8683.68	1,621.91	35,996.27	6,316.36	46,716.08	2,230.77	12,874.6	9,377.15	288.68	14,971.2	356.78	4,833.08
X13	E11	37	43,711.98	318.75	804.65	199.6	11747.98	1,761.37	29,334.05	1,867.19	47,412.71	1,126.15	12,418.37	20,018.83	358.07	13,564.16	409.94	3,506.51
X14	F11	38	27,590.74	215.92	2250.73	342.61	7151.07	2,600.25	71,506.34	2,221.52	143,849.22	1,836.12	10,373.1	10,614.41	971.48	8,672.85	880.42	2,944.15
X15	G11	39	23,369.32	227.39	836.49	201.87	5372.49	2,063.12	25,230.36	1,200.88	75,361.37	2,508.35	7,631.28	32,732.65	161.93	6,615.3	436.63	2,378.32
X16	H11	40	50,916.11	192.84	959.78	187	11139	1,973.63	40,374.23	1,391.47	48,640.63	1,890.85	11,044.04	56548.7	312.91	14,500.21	552.98	4,548.56
X17	A10	45	38,433.29	189.01	1425.32	133.48	8695.72	1,772.28	29,712.47	809.2	59,919.49	2,480.28	9,821.16	88,688.47	110.74	14,300.5	570.97	2,761.98
X18	B10	46	33,977.25	186.43	844.06	122.3	7197.6	2,222.78	31,156.84	925.44	81,600.29	4,576.02	11,408.04	7,895.59	239.63	11,262.29	216.51	3,047.61
X19	C10	50	30,534.22	232.97	355.78	187	8857.06	2,697.1	30,396.99	1,889.93	239,435.07	1,364.14	10,746.64	25,775.44	263.01	11,005.18	688.54	2,053.81
X20	D10	52	27,814.49	234.08	1,193.93	209.8	6,372.38	8,345.61	35,287.07	14,933.35	13,0974.97	2,614.29	9,231.67	19,699.15	289.15	5,193.67	634.15	3,437.34
X21	E10	53	41,194.32	213.57	1,585.51	160.08	11,778.68	1,652.04	25,503.75	8,867.08	43,257.69	1586.95,	8,120.18	14,051.31	251.79	12,848.56	472.31	2,048.89
X22	F10	56	51,383.15	213.57	893.82	137.17	11,071.46	2,117.29	20,954.76	1,493.47	49,721.54	3,045.12	17,738.76	30,736.99	206.79	16,220.73	561.97	4,125.68
X23	G10	57	45,314.2	201.56	588.44	144.48	6,380.63	1,580.78	34,209.81	4,757.91	46,876.29	1,872.79	11,984.85	7,957.41	226.98	9,582.25	392.19	3,506.51
X24	H10	58	45,500.66	183.83	1,849.83	166	10,494.5	1,734.07	21,640.72	1,138.35	34,311.51	1,725.73	11,408.04	62,363.57	282.15	9,893.64	494.66	4,289.66
X25	A9	59	12,906.9	181.19	297.23	94.05	8,888.71	2,014.33	36558.84	3,882.46	137,867.37	1,533.45	8,550.4	7,674.46	224.64	11,546.26	334.71	922.35
X26	B9	60	21991.3	265.72	318.1	146.9	11,828.68	2,249.8	20,980.71	967.11	32,612.32	3,040.13	12,296.42	11,063.17	203.97	13,687.45	277.58	5,218.71
X27	C9	62	42,998.1	205.22	605.74	149.31	6,270.97	1,794.1	40,168.33	7,798.95	81,497.34	2,220.31	10,085.24	9,829.68	204.91	15,367.97	409.94	2,619.29
X28	D9	63	28,452.32	194.1	658.82	151.72	6,457.91	2,022.46	35,455.99	6,342.25	47,231.87	4,262.59	10,366.86	25,486.26	254.6	15,618.73	418.83	1,980.04
X29	E9	66	30,449.97	206.43	1,016.45	177.73	13,996.49	1,951.9	48,721.76	1,402.97	102,051.06	2,435.49	12,479.29	6,229.72	171.89	14,868.17	543.99	3,180.71
X30	F9	67	50,829.86	204	890.81	144.48	10,271.58	1,984.48	19,248.9	1,080.61	34,535.76	4,376.61	13,323.3	13,669.24	169.52	165,50.51	720.35	5,359.31
X31	G9	69	34,868.32	208.83	1,382.99	122.3	6,570.45	962.45	26,104.44	759.45	219,173.74	2,320.86	13,951.93	6426.5	471.17	10,278.72	277.58	1,970.2
X32	H9	70	45,253.93	234.08	1,568.06	146.9	8,683.68	2,238.99	52,671.63	2,467.32	106,487.71	2,636.96	11,297.16	59,092.79	360.86	12,375.73	679.46	2,752.14
X33	A8	71	46,315.39	183.83	591.59	137.17	5,893.8	1,668.47	35,180.09	3,260.74	41,618.08	2,256.26	9,739.17	7,101.44	229.79	10,539.55	286.34	3,368.21
X34	B8	74	28,314.29	238.47	500.9	170.71	3,920.16	1,973.63	43,108.35	1,034.63	253,316.17	2,417.29	5,401.68	7,040.3	226.51	6,584.76	387.76	1,812.76
X35	C8	75	29,859.61	234.08	496.06	196.18	9,376.87	1,064.31	44,465.1	27,461.75	34,303.21	2,574.95	9,244.43	5,677.47	274.68	13,891.42	365.62	3,600.44
X36	D8	76	43,907.24	239.55	794.01	144.48	10,548.22	1,851.3	47,534.44	8,364.3	77,479.7	2,274.47	10,889.3	11,073.88	240.57	13,195.12	409.94	3,437.34
X37	E8	79	28,006.06	273.72	1,889.02	229.91	23,938.2	2,465.61	37,586.12	1,157.28	127,329.07	1,798.28	9,320.87	33,578.3	504.75	10,649.27	454.45	2,412.74
X38	F8	80	42,680.03	239.55	661.92	193.89	9,629.03	1,777.74	30,635.77	4,219.41	51,732.05	2,395.92	11,481.87	6,704.89	277.95	606.85	625.11	2,245.57
X39	G8	81	30,349.89	194.1	757.41	103.2	5,520.95	1,465.27	27,538.13	641.56	98,307.67	688.25	10,354.37	6,139.07	218.07	10675.4	308.29	1,798
X40	H8	82	30,492.09	139.24	1,229.21	151.72	9,535.82	1,799.55	43,893.83	1,725.59	58,992.25	1,535.06	12,552.37	24,127.25	330.14	11,141.54	490.19	2,029.22
X41	A7	83	35,439.93	204	1,387.37	158.89	10,410.68	1,140.2	24,940.79	5,314.59	155,799.26	1,280	9,498.76	10,486.64	314.31	11,577.85	242.62	2,786.59
X42	B7	84	15,883.99	201.56	751.29	129.78	9,572.2	1,613.69	46,180.18	7,492.91	75,025.73	1,749.41	8,113.62	15,297.74	178.51	12,603.94	347.94	2,771.83
X43	C7	85	46,735.77	231.87	1,645.11	146.9	11,761.4	1,404.54	38,871.09	1,273.91	205,445.21	626.26	15,324.57	13,796.41	506.15	13,843.08	347.94	3,175.78
X44	D7	87	37,115.56	202.78	1,252.71	149.31	7,273.53	1,498.32	23,625.2	1,276.91	54,819.6	2,023.13	7,677.85	28,390.53	151	16,991.8	445.54	1,674.9
X45	E7	88	37,715.83	204	437.5	185.84	9,563.73	1,421.12	38,442.45	11,108.99	76,849.34	729.16	10,104.06	3,582.1	199.26	7,697.32	321.49	3,136.33
X46	F7	89	41,761.44	204	769.63	160.08	7,551.31	2,122.71	31,171.46	1,321.52	106,440.19	2,075.67	8,238.02	13,873.91	267.22	10,863.74	494.66	2,737.38
X47	G7	90	52,189.8	230.75	3,042.06	229.91	23,836.51	6,177.42	38,087.13	1,144.68	156,570.71	1,621.92	8,679.8	24,561.58	370.16	10,529.11	526.03	3,008.19
X48	H7	91	51,852.4	225.13	455.51	139.61	11,803.66	1,602.72	24,847.13	4,681.42	31,002.89	3,028.61	13,613.7	18,688.21	286.35	10,518.67	334.71	4,051.21
X49	A6	92	37,326.08	212.39	258.27	133.48	8,130.39	2,103.76	29,213.96	5,619.88	25,209.03	2,868.61	6,930.2	8,687.5	173.78	20361.03	361.2	3,299.1
X50	B6	93	38,306.56	206.43	1,797.58	134.71	9,441.45	2,222.78	16,498.27	1,557.02	53,308.32	2,004.44	10,166.73	7,091.25	131.91	15,030.9	499.14	2,944.15
X51	C6	94	40,054.15	219.41	1,160.06	149.31	6,981.46	2,554.49	33,108.18	2,082.66	21,494.34	2,651.62	11,112.03	25,970.79	224.64	8,703.76	401.06	2,516
X52	D6	95	42,717.74	190.3	588.44	227.69	8,231.4	1,772.28	3,0884.14	882.78	67,511.47	2,310.65	9384.47	35,808.88	289.15	11,546.26	467.84	3,299.1
X53	E6	96	36,847.3	238.47	563.17	154.12	7,167.77	2,071.25	29797.46	7216.55	42,119.64	4,635.79	6,265.07	19,333.47	245.71	17,058.15	584.49	2,889.98
X54	F6	97	29,859.61	220.56	1,072.87	134.71	7,714	1,238.04	34,828.92	731.94	47,139.17	676.62	3,738.93	19,847.02	189.84	10,080.84	295.12	1,289.93
X55	G6	98	31,496.54	218.25	1,938.36	158.89	7,708.72	2,249.8	35,155.08	1,119.27	49,259.34	2,688.01	8,277.21	26,103.35	191.73	7,462.01	530.51	1,930.85
X56	H6	4	15,294.25	201.56	3,746.89	156.51	5,399.36	2,098.34	60,378.74	739.86	88,717.77	1,439.81	6,052.94	18,192.68	261.14	13,478.48	339.12	1,817.68
X57	A5	3	31,554.32	205.22	1,407.81	180.05	5,597.42	2,276.8	24,442.66	3,487.53	109,564.58	639.36	5,643.29	13,912.69	222.29	14,230.42	616.07	2,098.06
X58	B5	5	35,172.44	253.4	455.51	175.39	8,020.81	1,821.35	45,647.06	11,710.82	85,132.87	644.13	6,243.96	15,011.11	302.2	5,436.98	467.84	2,255.4
X59	C5	Control sample 1	44,494.53	211.21	333.6	158.89	10,657.94	1,285.36	25,941.41	2,779.34	47,495.94	996.4	5,910.24	16,767.86	231.2	11,462.05	303.9	3,200.43
X60	D5	Control sample 2	48,679.51	227.39	555.24	173.06	9,579.82	1,047.39	21642.37	21814.97	33,420.61	1,858.1	4,590.6	19,498.44	239.63	8,714.06	330.3	1,989.88
X61	E5	Control sample 3	48,837.34	221.71	705.23	161.27	9,520.62	1,114.95	27,611.22	25,687.62	50,480.96	1,523.18	5,283.23	13,299.87	234.01	8,066.29	273.2	2,599.62
X62	F5	Control sample 4	10,234.2	223.99	612.01	168.36	9,449.01	999.33	34,027.39	15,605.2	48,026.74	2,223.1	8,770.13	6,654.19	346.9	3,317.27	268.82	2,648.81
X63	G5	Control sample 5	51,048.51	257.55	558.41	200.74	6,943.48	1,613.69	32,300.62	1,462.65	63575.11	4,027.63	9,155.08	33,707.43	137.17	13,489.19	679.46	1,041.93
X64	H5	Control sample 6	43,495.32	196.61	629.22	149.31	7,246.47	1,371.35	24843.53	4,706.3	41,809.21	3,132.46	8,873.13	53,246.05	219	13,767.92	401.06	2,206.23
X65	A4	Control sample 7	57,370.41	308.83	621.41	156.51	14,562.36	1,832.24	50,724.18	14,183.94	51,299.12	2,234.6	12,996	22,147.93	311.98	19,355.92	277.58	4,061.14
X66	B4	Control sample 8	48,128.86	240.64	597.89	184.69	8,394.37	1,432.16	37,300.65	14,730.63	24,411.49	3,864.1	6,758.82	22,664.88	256.47	15,199.3	463.38	3,348.46
X67	C4	Control sample 9	35,193.41	157.58	452.25	168.36	8,849.77	1,098.09	25,432.49	9,376.29	42,532.41	896.39	7,336.84	14,301.4	231.2	11,888.97	607.04	2,757.06
X68	D4	Control sample 10	47,994.41	191.57	685.16	143.27	7,283.78	1,265.89	22,917.13	1,093.57	80,685.21	2,266.76	5,020.87	15,698.33	173.78	11,630.53	481.24	2,570.1
X69	E4	Control sample 11	42,760.84	259.61	839.52	231.02	4,957.36	1,547.83	43,824.16	19,957.61	68,355.45	2,831.43	9,657.05	7,756.67	248.52	11,377.9	729.45	1,640.41
X70	F4	Control sample 12	24,743.9	250.25	959.78	182.37	11,024.42	1,712.22	28,535.74	2,496.85	97,373.77	1,529.92	7,929.46	24,102.49	132.86	9,904.04	535	2,397.98
X71	G4	Control sample 13	38,264.33	249.19	435.86	203.01	8,610.06	1,679.41	32,427.09	5,056.3	57,058.31	2,039.13	5,708.59	20,446.78	258.81	16,958.64	383.32	3,101.82
X72	H4	Control sample 14	40,847.35	214.75	520.17	167.18	7,496.24	1,315.91	36,026.33	3,351.96	69,688.73	1,213.33	6,038.72	12,549.5	279.35	8,786.21	383.32	2,643.89
X73	A3	Control sample 21	17,149.31	251.3	780.31	205.28	8,575.77	1,695.82	38,287.46	1,359.61	44,960.73	2,105.84	11,960.38	21,839.1	324.56	17,501.53	570.97	5,088.33
X74	B3	Control sample 29	41,986.63	242.8	612.01	205.28	10,081.15	1,635.61	26,339.01	11,263.13	25,224	3,113.83	8,924.53	24,337.97	300.33	12,100.36	526.03	3,274.43
X75	C3	Control sample 28	43,160.07	211.21	497.68	188.15	7,428.04	1,310.36	24,913.75	16,109.13	54,808.85	2,385.26	8,067.67	48,159.58	247.12	13,649.91	454.45	2,471.74
X76	D3	Control sample 32	45,742.35	240.64	491.22	193.89	9,438.93	1,492.82	41,879.24	2,779.34	114,934.71	3,156.57	9,346.32	75,364.67	303.6	13,633.83	499.14	2,599.62
X77	E3	Control sample 33	39,783.29	220.56	726.76	196.18	8,973.42	1,630.13	29,639.75	15,369.4	45,050.89	2,970.87	6,321.27	23,233.68	238.69	19,463.35	588.99	2,176.73
X78	F3	Control sample 25	29,384.35	201.56	120.61	187	8,838.42	1,534.08	45,843.64	1,428.68	84,546.02	3,393.59	8,621.62	20,882.67	178.98	7,932.96	268.82	1,111.5