Original Article

Differential Immune Reactivity Pattern of SW48 and SW1116 Colorectal Cancer Cell Lines with Colorectal Cancer Patients Sera

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Abstract

Background: Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide. It is also known as the second leading cause of deaths as the early stage detection is not yet available by current methods. So identification of biomarkers can also be functional in early diagnosis and prognosis. **Materials and Methods:** We examined sera from 60 CRC patients of different stages as a source of auto-antibody as well as two human CRC cell lines with different invasive capacities (SW48 and SW1116) as the source of antigens. The pattern of immune reactivity in immuneblotting tests between mentioned cell lines and CRC patients' sera were evaluated by ImageJ software. **Results:** The Immune reactivity pattern of two cell lines (SW48 and SW1116) with CRC patients' sera were different in band intensities and the most immune reactivity intensity was observed in SW48 cell lysate with sera from Stage III CRC patients. **Conclusion:** Due to the humoral immune response, sera from Stage III CRC patients contained autoantibodies that demonstrated higher immune reactivity. Moreover, SW48 cell line with high aggressive behavior reacted to CRC patients' sera with greater intensity compared with less aggressive behavior cell line (SW1116). Therefore, it is required to use other techniques such as two-dimensional electrophoresis and mass spectrometry.

Keywords: *Auto-antibody, biomarker, colorectal cancer, immuno-blotting, SW1116, SW48*

Introduction

Colorectal cancer (CRC) as one of the most common malignant neoplasm in the world, results from uncontrolled cell growth in the colon, rectum or appendix.[1] It is obvious that early diagnosis is essential for survival. Fortunately, CRC death rates have reduced in recent years due to the early detection and treatment. However, the prognosis for this cancer remains poor.[1] Therefore, it is crucial to determine an effective method to detect signs of this disease in its earliest stages.[1] Many factors have been proposed as independent risk factors helpful in diagnosing, predicting the risk of recurrence and survival rates of cancer.[2-4] Good biomarkers for early diagnosis of cancer and prognosis are the ones that are present in the early stages of the disease, low or absent in normal subjects or patients with other diseases and easily quantifiable in the circulation.[5] Tumor-associated antigens (TAAs) from cancer cells along with potential tumor biomarkers in human malignancies are appropriate targets for humoral immune response induction.[6] Autoantibodies (aAbs) are a main part of the humoral immune response against tumor cells that react with TAAs and can be used as potential biomarkers in cancer immunodiagnosis and prognosis.[7] It is revealed that cancer patients' sera contain antibodies react with TAAs and can be utilized for potential therapeutic purposes, diagnostic and prognostic information of the disease.[8]

Serological proteome analysis is a high throughput technique that have been successfully used for proteomics analysis.[9] Antibodies against specific proteins in cell lysates (prepared from tumor tissues or tumor cells in their natural states) can be distinguished by proteomics-based approaches.[10] Endogenous proteins may also earn antigenic properties due to some variation such as changes in expression, posttranslational modifications, conformational changes, and other protein processing events.[11] Also using western blot technique as a part of proteomics approach can be helpful in identifying aAbs against tumor specific or associated antigens in the first step and design subsequent studies.

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Management of CRC treatment that has a powerful role in prognostic prediction is principally conducted by tumor stage.[12] It is indicated that an appropriate treatment is better to be selected on the basis of operational information, and the effectiveness of different treatments can be evaluated by comparing the survival rates after the treatments.[13] There exist various CRC screening methods that either through early stage detection of cancer or benign adenomas can decrease morbidity, mortality, and probably the incidence of CRC in the community.[14] Cancer stage detection is an essential factor for determining the treatment option and predicting the patients' prognosis. There is a commonly used system for CRC staging established by the American Joint Committee on Cancer which sometimes is called the tumor, node, metastasis (TNM) system.[15]

Several researches have been performed to identify helpful tumor markers in improving CRC diagnosis in early stages and to predict treatment response or survival outcomes.[16] Appropriate prognostic biomarkers could identify cancer predisposed individuals and facilitate appropriate treatment selection.[16]

The present study is aimed to compare the immunoreactivity pattern of sera from CRC patients (at different stages) and normal healthy individuals with two CRC cell lines (SW48 and SW1116) that had different invasive properties by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis - method. Based on western blot experiment results and immunoreactivity pattern, we realized that the maximum amount of aAbs in patient's sera is related to third stage of the disease also the highest intensity reaction was related to SW48 cell line with higher invasiveness properties. In addition, the lysate of several tumor cell lines such as breast cancer (MCF7 and SKBR3), lung cancer (A549 and MHR80) and pancreatic cancer (Miapaca2 and Patu8902) were used to assess the specificity of aAbs against tumor antigens that were present in CRC patients' sera. Of this study, outcome prompted us to design future studies using two-dimensional immunoblot techniques and mass spectrometry.

Materials and Methods

Patients' characteristics and serum collection

A total of 60 CRC patients including 15 in each Stage of I–IV were enrolled in the study. Samples were collected from Department of Colorectal Surgery, Faghihi Hospital, Shiraz, Iran. The research protocol was approved by the appropriate Ethics Committee, and all participants had signed written informed consent to be included in the study. Also, patients who had been treated before were excluded from the study. Demographic features and disease-related characteristics such as age, gender, and disease duration since the symptoms onset, were evaluated. Moreover, tumor pathological data of patients, including tumor type, histological grade, tumor size, tumor site, lymph node involvement ratio, metastasis and TNM staging were recorded. Fifteen normal healthy controls participated in this study as well (from Shiraz Institute for Cancer Research). Ten-milliliter blood from each individual was collected in 15-ml falcon tube. Then, the tubes were centrifuged, and serum was separated. Sera were frozen at − 80°C in aliquots of 200 µl in microcentrifuge tubes within 4 h after collection.

Cell culture

SW1116, SW48, MCF-7, SKBR3, A549, MHR80, MIAPACA2, and PATU8902 cell lines were obtained from National Cell Bank of Iran (NCBI, Pasteur Institute of Iran, Tehran, Iran). Cell lines were maintained in RPMI-1640 (AU565) (GIBCO, Invitrogen Corp., Carlsbad, CA, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco/BRL, Germany), 100 units/ml penicillin, and 100 µg/ml streptomycin (Biosera, The UK) under standard culture conditions (37°C, 5% CO_2, and 95% humidity). The culture media were changed every 2–3 days.

Invasion assays

Invasion assay was carried out using a 24 well Transwell insert (8 µm pore filters, BD Bioscience, Bedford, MA, USA) with the matrigel-coated membrane. Briefly, for invasion assays, the Transwell was covered with 100 µl (1 mg/ml) matrigel (BD Bioscience, Bedford, MA, USA) and was accumulated as an intervening invasive barrier. SW48 cells (5 × 103) and SW1116 cells (5 × 103) were plated onto the upper part of the matrigel-coated Transwell chamber and incubated for 24 h. After 24 h, the invaded cells were fixed with methanol, stained and counted. The number of invading cells were determined by calculating ten high-power fields (400) on each membrane and estimated as the mean number of cells per field.

Preparation of cell lysate

Cells were washed twice with ice-cold phosphate-buffered saline (PBS), scraped, centrifuged and then lysed in 50 mM Tris-HCL, 150 mM NaCl, 0.1% SDS, and 1 mM phenylmethanesulfonyl fluoride (PMSF, Sigma) plus protease inhibitor cocktail (Sigma). Samples were located in the dry block heater for 1 min at 98°C followed by high-speed centrifugation for a short time. The supernatant from each sample was subsequently collected, and protein concentration of the lysate was determined by Bradford protein assay using bovine serum albumin standards. Protein samples were stored at −80°C until needed for electrophoresis experiments.

Titer of IgG in serum samples

IgG concentration in serum samples was measured with the Abcam's IgG human ELISA (ab100547) kit as per the manufacturer's instructions. The intensity of the color is measured at 450 nm.

Sodium dodecyl sulfate electrophoresis and western blotting

Fifteen micrograms from each protein sample was diluted in loading buffer containing dithiothreitol (20 mM), denatured at 98°C for 5 min and loaded onto a 12.5% acrylamide gel (Merck). Electrophoresis was performed in running buffer (0.5 M Tris Base, 1.92 M glycine, 0.5% SDS) for 2 h at 90–110 V. After that, proteins were transferred to polyvinylidene fluoride (PVDF) membrane for Western Blotting (Roche Applied Science) on the ice at 100V 2 h using an electroblot system (BioRad, Hercules, California, USA). Then, the membrane was blocked in a maximum blocking time of 2 h in room temperature with blocking buffer (5% milk in Tris-buffered saline, 0.05% Tween 20), followed by 4 times washing with PBS-Tween buffer. The membrane was incubated with serum as primary antibody (1:70 dilutions) overnight at 4°C. Washing gently 4 times in PBS-Tween, the membrane was subsequently incubated for 1.5 h at room temperature with secondary goat anti-human IgG-peroxidase antibody (sigma) at a dilution of 1:1500. Mouse anti-beta-actin antibody (Abcam) was used as an internal control for western blot. After additional washing with PBS-Tween, each blot was finally developed using diaminobenzidine tetrahydrochloride (Sigma) substrate [Figure 1].

Software-based analysis of western blots

After immunodetection step, blots were scanned in a GS-800 Calibrated Densitometer (Bio-Rad) and digital images were analyzed by ImageJ software. Each blot was analyzed in a semi-automated way, and background intensity was subtracted from each sample. Inside each band, an equal area (area of interest) was chosen, and data were normalized to values of actin band as a control. The ratio related to each band of interest was quantified versus actin band and was counted by ImageJ software (NIH, Bethesda, MD, USA) [Figure 2]. Three independent Western blots were carried out for each stage and normal healthy controls. Furthermore, to determine the specificity of the reaction, the band intensity of CRC patients' sera in reaction with SW48 and SW1116 cell lines lysate were compared with other tumor cell lines (derived from different cancer tissues).

Statistical analysis

Data were analyzed using nonparametric analysis of variance and Bonferroni posttests, to investigate the intensity of immunoreactive bands among four groups (Stage I, Stage II, Stage III and Stage IV). All data are shown as mean ± standard error.

Results

SW48 cell line has higher invasion strength from SW1116 cell line

To evaluate the potency invasion activity between SW48 and SW1116 CRC cell lines, we conducted *in vitro* Transwell matrigel coated Transwell invasion assay. As shown in Figure 3, the number of invaded SW48 cell

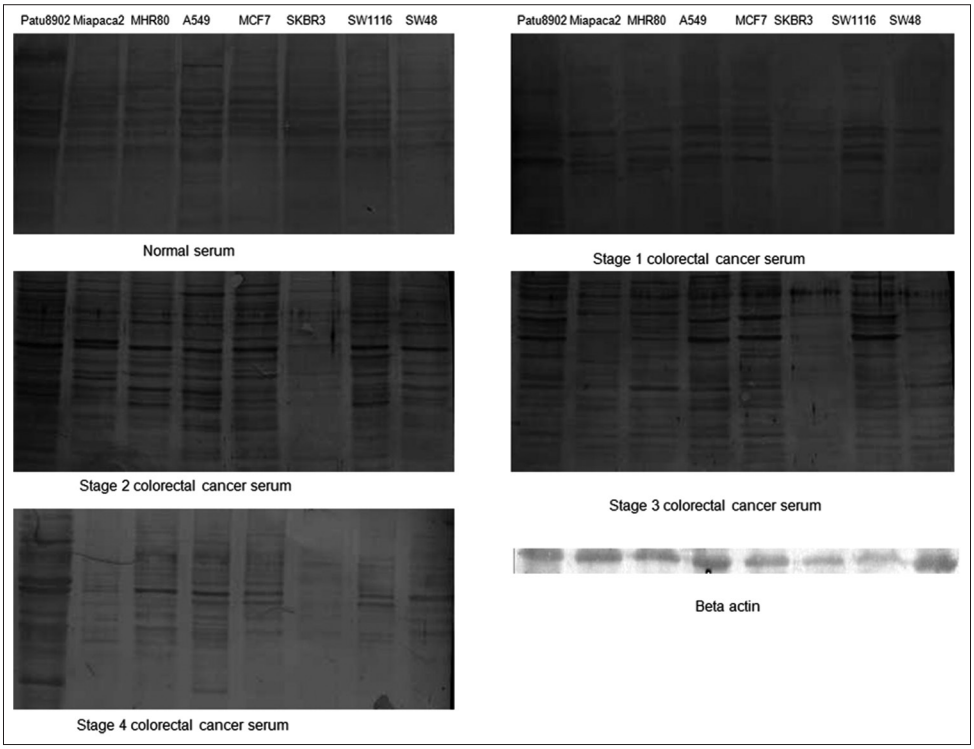

Figure 1: Immunoblot image of normal healthy controls and patients' (I–IV Stages of colorectal cancer) sera reactions with eight cell lines lysate. All of experiments were run in triplicate and Beta actin was used as control

line significantly higher in compared to the SW1116 cell line ($P < 0.02$).

IgG level in serum samples

Previous to performing additional examinations of the differences seen in the immune reactivity pattern between various patients' subsets and extracted cells lysate, we first required to eliminate the possibility that the differences seen by western blotting were just a reflection of important gaps in the quantity of total IgG existing in the sera tested. Consequently, we measured the level of total IgG in all samples. According to the results, we cannot find significant differences in serum IgG titer between the individual samples [Supplement Table 1].

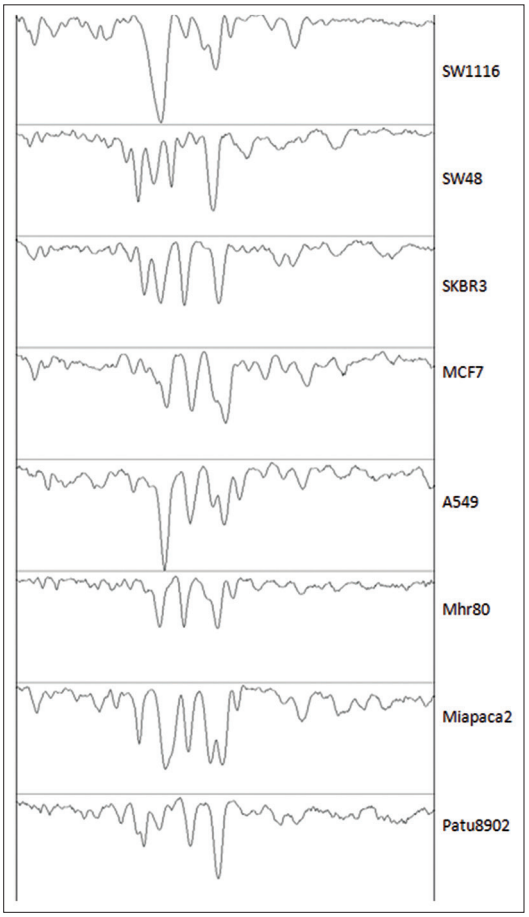

Figure 2: The graph of band intensities from the reaction of eight cell line lysate with colorectal cancer patients' sera created by ImageJ software. For each blot a graph was plotted by ImageJ software and the intensities of the bands were quantified by measuring the area under the curve

Sera from colorectal cancer patients show different immune reactivity to SW48 cell lysate compared with normal serum

Cancer patients' sera may contain antibodies that are specific for TAAs and can be used for disease detection. As a result, antibodies present in the serum of CRC patients might be considered as diagnostic and prognostic biomarkers in CRC. Patients were classified according to clinical and pathological data, and their sera were collected. Lysates of different cell lines were incubated with patients' sera for western blot analysis. For instance,

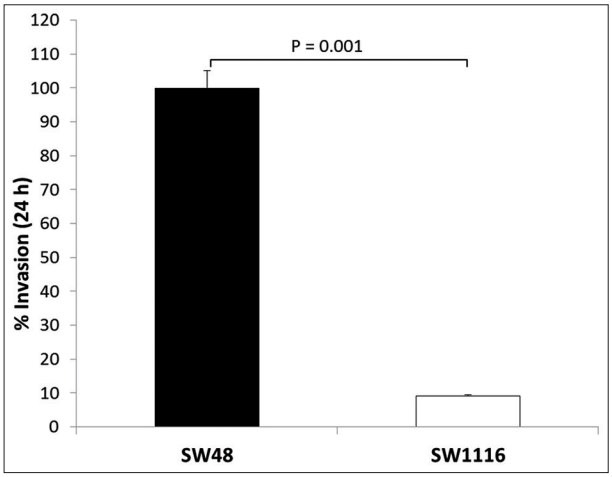

Figure 3: A fixed number of cells were plated onto the upper part of the matrigel-coated Transwell chamber. After incubation for 24 h, invasive cells were counted at the lower part of Transwell

Table 1: Comparison of the mean intensity of immune reactive bands between SW48 and SW1116 cell line lysates in reaction with CRC patients' sera

Immune reactivity band	SW48 vs SW1116				
	Normal sera	CRC Stage I Sera	CRC Stage II Sera	CRC Stage III Sera	CRC Stage VI Sera
Band 1 mean intensity	0.77 vs 0.61	0.86 vs 0.67	0.84 vs 0.69	1.08 vs 0.79	0.83 vs 0.76
Band 2 mean intensity	0.5 vs 0.63	0.48 vs 0.68	0.76 vs 1.05	1.28 vs 0.79*	0.56 vs 0.63
Band 3 mean intensity	0.7 vs 0.7	0.77 vs 0.7	0.92 vs 1.06	0.99 vs 0.7	0.73 vs 0.74
Band 4 mean intensity	0.93 vs 0.99	0.89 vs 0.98	1.23 vs 1.1	1.75 vs 0.62**	1.08 vs 0.7
Band 5 mean intensity	0.89 vs 0.73	0.88 vs 0.73	1.31 vs 1.08	1.44 vs a. 63**	0.85 vs 0.86
Band 6 mean intensity	0.99 vs 1	1.01 vs 1.02	0.99 vs 0.72	1.87 0.81***	1.42 vs 1.24
Band 7 mean intensity	1.23 vs 1.1	1.31 vs 1.1	1.6 vs 1.5	1.2 0.59**	1.06 vs 0.59*
Band 8 mean intensity	0.97 vs 0.63*	1.02 vs 0.63*	1.72 vs 0.57**	2.03 vs 1.07***	1.67 vs 1.15*
Band 9 mean intensity	1.07 vs 1.16	1.22 vs 1.21	1.31 vs 1.13	0.65 vs 0.49	1.06 vs 1.11
Band 10 mean intensity	1.03 vs 0.91	1.18 vs 0.97	1.05 vs 1.06	0.95 vs 0.6	1.07 vs 0.99

*P<0.05, **P=0.01, ***P=0.001. CRC: Colorectal cancer

SW48 cell lysate on PVDF membrane was treated with normal sera and sera from patients with Stages I–IV CRC. As it was mentioned, immune blotting results were analyzed by ImageJ software and 10 bands from each lane were selected for further evaluating of the density. The molecular weights of these bands were selected between size ranges of 25–110 kDa. Results from these analyses are shown in Figure 4. Among 10 reactive bands obtained from SW48 cell line, 4 bands (bands 2, 4, 6 and 8) in patients had a significantly higher intensity ($P < 0.001$) than normal ones and the most intensity was related to sera from patients with Stage III CRC. Furthermore, the intensity of bands 6 and 8 in Stage IV of CRC patients were significantly ($P < 0.001$) elevated in relation to normal sera. Among patients with Stage II of CRC only the intensity of band 8 was significantly ($P < 0.001$) higher than band 8 from normal sera and the other bands, intensities were not significantly different. Also, no significant difference was detected between the bands obtained from SW48 cell lysate and sera of patients with Stage I compared with normal serum.

Immune reactivity pattern of patients and normal sera with SW1116 cell lysate

Immune reactivity of SW1116 cell lysate with different CRC patients' sera (Stages II–IV) revealed that 5 from 10 bands had different intensity in relation to normal controls' sera [Figure 5]. There was not any immune reactivity difference between normal and Stage I CRC patients' sera. Bands 2, 3 and 7 related to Stage II patients' sera in reaction with SW1116 cell line lysate had a significantly higher ($P < 0.01$) intensity in relation to normal controls reactions. Moreover, B and 4 in Stage III and 8 in Stage IV of patients showed more robust immune reactivity ($P < 0.01$) compare with normal serum.

Comparison of immune reactivity against lysates from SW48 versus SW1116 cells

The immune reactivity pattern of patients' sera (from different stages of CRC) with SW48 and SW1116 cell lysate were different [Table 1]. However, it was similar for normal control sera, and no significant difference was seen, except for band 8 which had a higher intensity in SW48 cell lysate. Immune reactivity pattern between both Stage I and Stage II patients' sera with SW48 and SW1116 cell lines lysate showed that the only difference was in band 8 intensity. However, for both groups the band 8 Intensity related to SW48 cell lysate was higher than SW1116 that had the $P < 0.05$ and $P = 0.01$, respectively.

The most difference in immune reactivity pattern exists between SW48 and SW1116 cell line lysate in reaction with sera from Stage III CRC patients as the band intensity was extremely clear. Therefore, both bands 6 and 8 showed significant differences in intensity ($P = 0.001$) in both cell lines. Moreover, the intensity of bands 4, 5 and 7 related to SW48 cell lysate were higher than SW1116 cell lysate ($P = 0.01$) as well as the intensity of band 2 from SW48 cell lysate which was also significant ($P < 0.05$) in compared to SW1116 cell lysate. The immune reactivity pattern of Stage IV patients' sera with SW48 and SW1116 cell lysate also confirmed that from 10 bands only bands 7 and 8 were different among two cell lines. The mean intensity of band 7 and 8 in Stage IV patients' sera in reaction with SW48 cell lysate were significantly higher than that of SW1116 cell lysate ($P < 0.05$).

Comparing the immune reactivity of colorectal cancer patients' sera with SW48 and SW1116 versus MCF7, SKBR3, A549, MHR80, MIAPACA2 and PATU8902

The ImageJ software was used to elicit the signal intensities of each immunoblot page and generate a plot diagram. The

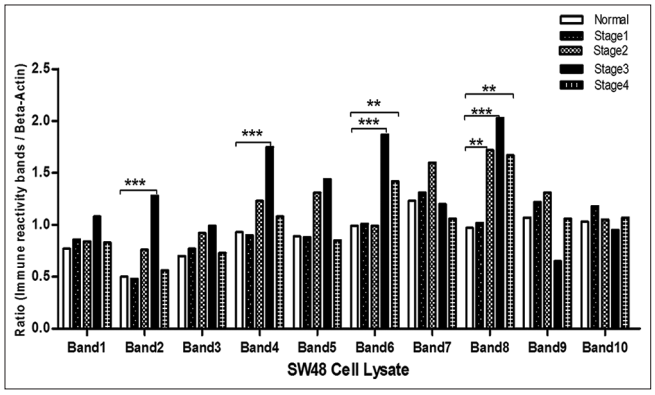

Figure 4: The mean intensities of 10 immune reactive bands relating to normal and four stages of colorectal cancer patients' sera reacted with SW48 cell lysate. According to the immunoblotting evaluation by ImageJ software 10 bands (that were common in all serum immune reactivity) were selected for calculating the mean intensity. Among these 10 reactive bands, 2, 4, 6, and 8 had a significantly higher intensity ($P = 0.001$) in Stage III patients' sera versus normal ones. Moreover, the most difference intensity was related to sera from patients with Stage III and normal ones

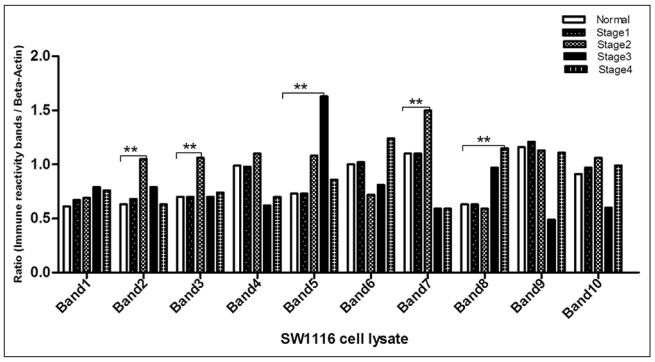

Figure 5: The mean intensities of 10 immune reactive bands relating to normal and four stages of colorectal cancer patients' sera reacted with SW1116 cell lysate. There was not any significant difference in immune reactivity pattern of normal and Stage I colorectal cancer patients' sera. Yet, there was a significant difference in the intensity of bands 2, 3 and 7 of sera from stage II colorectal cancer patients in reaction with SW1116 cell lysate versus normal sera ($P < 0.01$). Besides, the intensity of band 4 and 8 in Stage III and IV respectively showed significant differences in comparison with normal sera ($P < 0.01$)

intensity of each graph was analyzed by considering the reactions between different stages with the different cell line lysate. Although the degree of intensity was totally different between cell lines lysate and serum from I to IV Stages of CRC patients, we were unable to find a unique band specifically relating to one special cell line lysate or specific stage of CRC.

Discussion

It is a long period that researchers follow-up various studies for early detection of CRC by using an affordable, convenient, and noninvasive method. More generally serum biomarkers are one of the useful tools that may offer insight into prognosis, provide diagnostic plan or used for monitoring the treatment response, blood samples are practically easy to obtain and contain antibodies generated against tumor antigens that may serve as a promising source of biomarkers.

Albeit there has been an improvement in current knowledge of CRC molecular pathogenesis in the last two decades, still reliable and robust biomarkers are lacking to allow screening, surveillance, and primary prevention of this disease.[17] Therefore, diagnosis and treatment of CRC continue to be dependent on descriptive classification and staging systems which are mainly based on morphology and histology.[17] Utilizing humoral immunity may help us to find biomarkers for detection, prognosis and treatment of CRC patients. In fact, humoral immune response results in the production of aAbs against tumor antigens. CRC patient's sera contain aAbs that react with cancer associated or cancer-specific antigens.

In this study, we found that the immune reactivity patterns of CRC patient's sera of distinct disease stages with two CRC cell lines (SW48 and SW1116) are different from the pattern of normal sera in reaction with the same cell lines. The results confirmed that the most level of aAbs was present in sera from Stage III CRC patients that react with SW48 cell lysate. According to TNM staging system of bowel cancer, Stage III of CRC, has extended outside the colon to one or more nearby lymph nodes but no cancer spread to any other part of the body is observed. Although Suppiah *et al*.,[18] reported that there is no significant difference in aAbs against a human p53 protein in different stages of the CRC patients' sera. However, Kojima *et al*.,[19] described that the production of aAbs against markers such as carcinoembryonic antigen (CEA), p53 and CEA-IgM complexes were different at I–IV Stages of CRC patients and the highest aAbs level was measured in Stage III of disease. Also, Lechpammer *et al*. suggest that the existence of aAb against p53 in CRC patients' sera is a sign of tumors with more advanced histopathologic stages (Dukes' B, C).[20]

One possibility is that in the regional lymph nodes, TAAs or tumor specific antigens stimulate the immune responses. While the immune system response may be induced before the presence of tumor antigens in lymph nodes, our study proved that most humoral immune response occurs in Stage III of CRC disease. Furthermore, the present results illustrated that SW48 cell lysate in reaction with a different stage of CRC patient's sera has higher intensity bands than SW1116 cell lysate.

SW48 cell line has higher invasive properties when compared with the SW1116 cell line.[21] High invasive properties are associated with expression variation of certain molecules like matrix metalloproteinase (MMP2) and MMP9.[21] Therefore, an increased expression of some proteins (related to SW48 invasiveness) during invasion/migration process may stimulate an immune response. Sera from Stage III CRC patients contain aAbs that react with tumor cell lines lysate and our results demonstrated that intensity of immune reactivity bands with SW48 cell line was significantly higher than SW1116. This may be because tumor cells migration to lymph node occurs due to the expression of some proteins involved in invasion/migration process. SW48 cell line has such proteins; consequently, it shows the highest intensity in immune reactive bands. In recent years, many studies have been conducted to find aAbs against TAAs. Also numerous aAb markers with good diagnostic efficacy have been determined which can distinguish healthy controls from CRC patients.[22]

The present study is our first investigation in screening candidate tumor antigens in CRC and additional studies on tumor tissues are required that might identify other antigens that elicit humoral immune responses. Also, through further studies these antigens need to be identified by high-throughput techniques.

Due to the humoral immune response that reacted with infiltrated tumor cells into the lymph node, sera from Stage III CRC patients contained aAbs that demonstrated higher immune reactivity. Moreover, SW48 cell line with high aggressive behavior reacted to CRC patients' sera with greater intensity compared to less aggressive behavior cell line (SW1116).

Conclusion

We have identified the presence of aAbs with high immune reactivity properties in Stage III CRC patients sera that reacted with protein extraction from SW48 cell lines. Therefore, it is required to use high throughput techniques such as two-dimensional electrophoresis and mass spectrometry so that differences in the immunoblotting pattern of CRC patients' sera and cell lines could be identified with more details.

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Conflicts of interest

There are no conflicts of interest.

References

1. Cheng YL, Zhang GY, Li C, Lin J. Screening for novel protein targets of indomethacin in HCT116 human colon cancer cells using proteomics. Oncol Lett 2013;6:1222-8.
2. Zlobec I, Lugli A. Prognostic and predictive factors in colorectal cancer. Postgrad Med J 2008;84:403-11.
3. Compton CC. Colorectal carcinoma: Diagnostic, prognostic, and molecular features. Mod Pathol 2003;16:376-88.
4. Turner RR, Li C, Compton CC. Newer pathologic assessment techniques for colorectal carcinoma. Clin Cancer Res 2007;13 (22 Pt 2):6871s-6.
5. Pionneau C, Canelle L, Bousquet J, Hardouin J, Bigeard J, Caron M. Proteomic analysis of membrane-associated proteins from the breast cancer cell line MCF7. Cancer Genomics Proteomics 2005;2:10.
6. Kulasingam V, Diamandis EP. Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. Nat Clin Pract Oncol 2008;5:588-99.
7. Grandjean M, Dieu M, Raes M, Feron O. A new method combining sequential immunoaffinity depletion and differential in gel electrophoresis to identify autoantibodies as cancer biomarkers. J Immunol Methods 2013;396:23-32.
8. Hamsher C, Smith AM, Dehqanzada ZA, Khoo S, Ponniah S, Peoples GE, *et al.* Pattern of serum immunoreactivity against breast cancer cell lysates may predict severity of disease in breast cancer patients. Cancer Immunol Immunother 2007;56:1711-21.
9. Carr KM, Rosenblatt K, Petricoin EF, Liotta LA. Genomic and proteomic approaches for studying human cancer: Prospects for true patient-tailored therapy. Hum Genomics 2004;1:134-40.
10. Gao H, Zheng Z, Mao Y, Wang W, Qiao Y, Zhou L, *et al.* Identification of tumor antigens that elicit a humoral immune response in the sera of Chinese esophageal squamous cell carcinoma patients by modified serological proteome analysis. Cancer Lett 2014;344:54-61.
11. Soussi T. p53 Antibodies in the sera of patients with various types of cancer: A review. Cancer Res 2000;60:1777-88.
12. Greene FL, Sobin LH. The staging of cancer: A retrospective and prospective appraisal. CA Cancer J Clin 2008;58:180-90.
13. Compton CC, Greene FL. The staging of colorectal cancer: 2004 and beyond. CA Cancer J Clin 2004;54:295-308.
14. Neely D, Campbell W, Davey P, Rodgers C, McCrory D. Colorectal cancer screening: The northern trust experience. Ulster Med J 2013;82:160-3.
15. Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, *et al*. Screening and Surveillance for the Early Detection of Colorectal Cancer and Adenomatous Polyps, 2008: A Joint Guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 2008;58:130-160.
16. Clarke SJ, Karapetis CS, Gibbs P, Pavlakis N, Desai J, Michael M, *et al.* Overview of biomarkers in metastatic colorectal cancer: Tumour, blood and patient-related factors. Crit Rev Oncol Hematol 2013;85:121-35.
17. Nambiar PR, Gupta RR, Misra V. An "Omics" based survey of human colon cancer. Mutat Res 2010;693:3-18.
18. Suppiah A, Alabi A, Madden L, Hartley JE, Monson JR, Greenman J. Anti-p53 autoantibody in colorectal cancer: Prognostic significance in long-term follow-up. Int J Colorectal Dis 2008;23:595-600.
19. Kojima T, Yoshikawa K, Matsui T, Kodera Y, Kojima H. Titration of serum CEA, p53 antibodies and CEA-IgM complexes in patients with colorectal cancer. Mol Med Rep 2009;2:477-80.
20. Lechpammer M, Lukac J, Lechpammer S, Kovacevic D, Loda M, Kusic Z. Humoral immune response to p53 correlates with clinical course in colorectal cancer patients during adjuvant chemotherapy. Int J Colorectal Dis 2004;19:114-20.
21. Roh SA, Choi EY, Cho DH, Jang SJ, Kim SY, Kim YS, *et al.* Growth and invasion of sporadic colorectal adenocarcinomas in terms of genetic change. J Korean Med Sci 2010;25:353-60.
22. Chen H, Werner S, Tao S, Zörnig I, Brenner H. Blood autoantibodies against tumor-associated antigens as biomarkers in early detection of colorectal cancer. Cancer Lett 2014;346:178-87.

Supplement Table 1: Level of IgG in sera of healthy and patients subjects

IgG level in different groups of subjects sera on the basis of mg/dl				
Healty subjects	**Stage I CRC patients**	**Stage II CRC patients**	**Stage III CRC patients**	**Stage VI CRC patients**
1108	965	1206	895	1103
1134	1097	884	987	1102
997	986	1126	1041	979
1006	1293	1041	1252	768
1066	1115	983	959	1136
986	1056	929	1146	1221
1096	906	971	1098	1136
1016	1069	1140	976	965
1035	913	877	1045	891
945	1135	1064	1223	886
1330	1068	968	1007	1058
1094	1102	1071	884	1073
1151	1081	1245	1159	982
798	953	1181	1037	1054
1087	1166	960	1223	1247

CRC: Colorectal cancer