

The Relationship between the Intercellular Adhesion Molecule-1 Expression and the Response to BCG Immunotherapy in Non Muscle Invasive Bladder Cancer

Faouzia Ajili^{1*}, Sonia Abdelhak², Amel Nedri¹, Nadia Kourda³, Afifa maaloul¹, Salma Karay¹, Mohamed Slim Salmi⁴ and Samir Boubaker¹

¹Laboratory of Human and Experimental Pathology, Pasteur Institute of Tunis, Tunisia

²Laboratory of Medical Genetics and Oncogenetics, Pasteur Institute of Tunis, Tunisia

³Department of Pathology, Charles Nicolle Hospital, Tunis, Tunisia

⁴Department of Urology, Charles Nicolle Hospital, Tunis, Tunisia

*Corresponding author: FaouziaAjili, Laboratory of Human and Experimental Pathology, Pasteur Institute of Tunis, Tunisia, Institut Pasteur de Tunis, 13, Place Pasteur, 1002 Tunis, Tunisia, Tel: 00 216-71-789 608; Fax: 00 216-71-791 833; E-mail: Faouziaajili@yahoo.fr

Rec date: Apr 18, 2014, Acc date: Jul 05, 2014, Pub date: Jul 07, 2014

Copyright: © 2014 Ajili F, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Background: Bladder cancer is the second most common malignancy of the urogenital region. Although Bacillus Calmette-Guerin (BCG) is considered as the adjuvant treatment of choice for non-muscle invasive bladder cancer (NMIBC), there is no consensus for a predictive factor to assess BCG success. The intercellular adhesion molecule-1 (ICAM1) has been reported to function in multiple malignancies, but its effect on NMIBC hasn't been discussed yet. This study attempted to evaluate if ICAM-1 could be useful predictive markers in BCG responses.

Materials and Methods: Thirty primary resected NMIBC patients were included in the study. All patients received adjuvant BCG instillations. ICAM1 expression was inspected by immunohistochemistry and correlated with clinicopathologic variables. Association between protein expression and BCG therapy response was evaluated by univariate and multivariate analysis.

Results: Univariate Cox regression analysis of baseline characteristics and ICAM-1 expression showed that no significant association was found with BCG immunotherapy response. In the other hand, multivariate Cox regression analysis showed that ICAM-1 protein is not an independent factor of tumor response after BCG immunotherapy.

Conclusions: this study demonstrates that ICAM1 could not be a useful prognostic marker for BCG treatment in NMIBC. We emphasize that this was a preliminary study and therefore further confirmation on a larger set of tissues is necessary.

Keywords: BCG; Non muscle invasive bladder cancer; Adhesion

Introduction

Non-muscle-invasive bladder cancer (NMIBC) (Ta, T1, Tis stages) treated by transurethral surgery has high recurrence potential (60–70% of cases) and a risk of invading muscle in 15–25% of patients [1]. Intravesical instillations of Bacillus Calmette-Guerin (BCG) are the most effective adjuvant treatment for preventing recurrence and progression [2]. The failure of this local immunotherapy requires a more aggressive surgery. Therefore, the assessment of the factors correlated with high risk of tumor recurrence after BCG therapy is an important step guiding the therapeutic protocol in NMIBC [3,4]. Unfortunately, predictive markers for recurrence and progression are lacking. Prediction of recurrence or progression would be of great clinical benefit.

Currently, tumor grade and stage are the major prognostic factors. Efforts have been made to identify other potential prognostic markers that may better stratify and identify the true malignant potential of bladder cancer. Actually, there is no well-established predictive factor of the response to intravesical instillation of BCG and the mechanism of the anti-tumor effect of BCG therapy is not very clear. A biologic marker could help predict failure in refractory patients and allow early

cystectomy before aggressive superficial carcinoma occurs. In fact, a delay in appropriate therapy may lead to metastatic dissemination and death. Intercellular adhesion molecule-1 (ICAM-1, CD54) is a single chain cell surface glycoprotein that is constitutively expressed or induced on the surface of different types of cells. [5-7] ICAM-1 serves as a counter-receptor for the leukocyte integrin lymphocyte function-associated antigen (LFA-1). It mediates binding to the integrin lymphocyte function-associated antigen present on leukocytes and is particularly important for the attachment and subsequent transendothelial migration of leukocytes [8]. In fact, these proteins are among the most immunogenic reported molecules. However, their expression on activated endothelial cells and on various other cell types is markedly induced during inflammation. Because BCG therapy is immune dependent, the activation of a Th1 immune response could be required for clinical efficacy; therefore, the role of ICAM-1 protein in humoral response stimulated by BCG warrants investigation. The aim of this study was to investigate the prognostic and predictive value of ICAM-1 immunohistochemical expressions in a retrospective series of NMIBC before treatment, and to assess its correlation with BCG-immunotherapy response.

Materials and Methods

Cohort of Patients

Our retrospective study includes 30 patients enrolled between 2006 and 2008 at the Department of Urology in Charles Nicolle Hospital, Tunis, Tunisia. Inclusion criteria were cystoscopically and histologically confirmed NMIBC including Ta and T1 stages. No cases of carcinoma in situ were found in our study. Other exclusion criteria were upper urinary tract tumors, urinary tract infections, and other neoplastic diseases. All patients were informed about the aim of the study and signed a written consent stating their agreement to participate in the trial. The present study was also approved by the local research ethical committee of the Pasteur Institute of Tunis. The initial tumor, sampled immediately before BCG treatment was used for this purpose. Patients underwent complete transurethral resection, and the muscle layer was always assessable. Transitional cell carcinomas of the bladder were treated for the first time with 6 weekly instillations of intravesical BCG (BCG Pasteur strain, 75 mg in 50 mL saline), 3-6 weeks after the last transurethral resection. After the last instillation, urinary cytology and cystoscopic examination with randomized sites of mapping cold biopsies were performed. If these examinations were negative, patients continued the maintenance treatment, which consists of 24 additional monthly instillations. The follow-up was performed for 26 months. The follow-up time was calculated as the number of months from the date of the surgical procedure to the last cystoscopic control or the last visit. Patients underwent urine cytology and cystoscopy every 3 months during the follow-up. Responders to BCG immunotherapy were defined as patients who did not show cystoscopic or cytological evidence of tumor recurrence during the 26 months. The present study was also approved by the local research ethical committee of the Pasteur Institute of Tunis which is in agreement with Helsinki declaration. Recurrence was defined as reappearance of tumor after the initial treatment with at least one tumor-free cystoscopy interval. The endpoint for follow-up was either development of recurrent cancer tumor or the termination date of the study. Follow-up results were recorded and used for univariate and multivariate analysis.

Clinical and Histological Data

The resulting grade and stage were evaluated according to the 2004 WHO grading system [9] and TNM 2002 revision. For each patient, data were collected on tumor size, number of tumor loci, histological grade (low grade and high grade) and stage (pTa or pT1).

Immunohistochemistry

The specimens for immunohistochemical analysis were formalin-fixed and paraffin-embedded. One 4mm section from each patient was cut and put on silanized microscope slides (Dako, Copenhagen, Denmark). Tissues sections were deparaffinized, and heat-induced antigen retrieval was performed in 0.01 M citrate buffer, pH 6.0. They were then treated with 0.03% hydrogen peroxidase for 5 min to block endogenous peroxidase activity. They were washed 3 times in PBS for 5 min, incubated for 5 min with proteinblock, and then washed 3 times in PBS for 5 min. All the specimens were incubated at room temperature for 1 h with mouse monoclonal primary antibodies: anti-ICAM-1 (clone 23G12, 1:10 dilution, Leica). Sections were rinsed 3 times in PBS for 5 min, incubated for 20 min with secondary antibody, and then washed 3 times in PBS for 5 min. The detection was

performed using NovoLink TM polymer (Novocastra) for 15 min and 3,3'-diaminobenzidine chromogen. Sections were counterstained with hematoxylin, dehydrated, and mounted.

Semi-quantitative and Qualitative Assessment of Immunostaining

Microscopical analysis using an optical microscope (Zeiss, Axioskop) was performed by two pathologists. ICAM-1 expression was evaluated according to the proportion of positively stained cells and their exact localisation. For each tumor, the percentage of immunostained cells was assessed in at least 10 optical fields under high-power magnification (G×400). ICAM-1 staining intensities was rated on a scale of 0-3 according to the percentage of positive tumor (0.5% positive cells; 1, 5–20%; 2, 20–50%; or 3, .50%). The expression is very low for 0, low for 1, moderate for 2 and high for 3. ICAM-1 expression was classified as low for scores ≤ 1 (Figure1) and high for scores ≥ 2 (Figures 2 and 3).

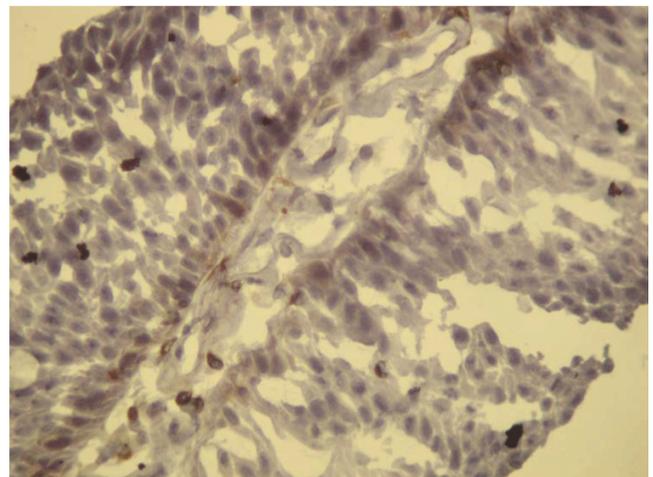


Figure 1: Low ICAM-1 positive immunostaining.GX400

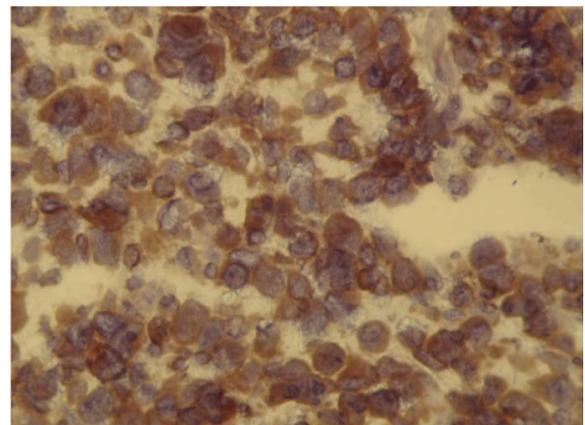


Figure 2: High ICAM-1 positive immunostaining.GX1000

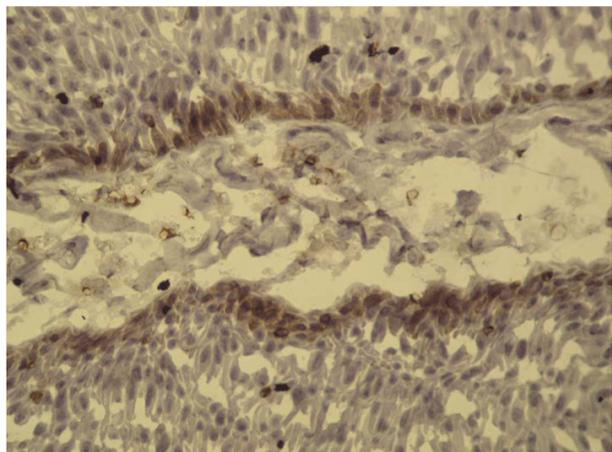


Figure 3: High ICAM-1 positive immunostaining. GX400

Statistical Analysis

SPSS for Windows (17.0 SPSS, Inc. Chicago, Illinois, USA) was used for statistical analysis. Frequency tables were analyzed using the Chi-square test, with Fisher's exact test to assess the significance between categorical variables. In addition, Cox regression was used to compute the hazard ratio (HR) attributed to ICAM-1 expression, categorized, while adjusting for age, sex, grade, loci number, tumor size, and stage. A multivariate Cox regression was used to select the best predictive model based on the AIC criteria as well as some biological considerations. All reported p values were two-sided and statistical significance was considered as p less than 0.05.

Results

Host and Tumors Characteristics

Our study included 28 men and two women. The median age was 60 years (range, 25-80). 13 patients (43.3%) were aged less than 60 years and 17 (56.6%) were aged over 60 years. Tumors were multiple in 36.7% of cases (n=11) and tumor size was more than 3 cm in 16 patients (53.3%) (Table 1). The 30 studied tumors were subdivided into 14 pTa stage (46.7%) and 16 pT1 stage (53.3%). All patients were alive at the end of the follow-up period and no patient was lost to follow-up. The half of patients (50%) had experienced at least one recurrence.

The Association of ICAM-1 with Clinicopathological Variables

ICAM-1 staining mainly located in cytoplasm of tumor cells. Expression of ICAM-1 was low in 19 of patients (63.3%) and high in 11 patients (36.6%). According to the results of immunohistochemistry, we correlated ICAM-1 status in 30 NMIBC specimens with other widely recognized clinicopathologic parameters (Table 1). Our analyses showed that no significant association was observed between ICAM-1 positive expression levels and the clinicopathologic factors. However, all the patients with high ICAM-1 expression had experienced at least one recurrence.

Variable			ICAM-1 expression		P Value
	Low		High		
	n/N	%	n/N	%	
Sex					
Female	0	0	2	100	0.52
Male	11	39.3	17	60.7	
Age, Yr					
<60	6	46.2	7	53.8	0.513
≥ 60	5	29.4	12	70.6	
Tumor size					
<3 cm	3	21.4 s	11	78.6	0.142
≥ 3 cm	8	50	8	50	
No. of Tumor					
Single	6	31.6	13	68.4	0.696
Multiple	5	45.5	6	54.5	
Grade					
Low grade	9	39.1	14	60.9	0.314
High grade	2	28.6	5	71.4	
Stage					
pTa	5	35.7	9	64.3	0.293
pT1	6	37.5	10	62.5	

Based on Pearson χ^2 test or Fisher exact test. p<0.05 is significant.

Table 1: Association between ICAM-1 expression in NMIBC with baseline characteristics

Relationship between ICAM-1 expression and BCG immunotherapy response

Univariate Cox regression analysis of baseline characteristics and ICAM-1 expression showed that no significant association was found with BCG immunotherapy response (Table 2). In the other hand, multivariate Cox regression analysis showed that ICAM-1 protein is not an independent factor of tumor response after BCG immunotherapy (Table 3).

Predictive Event factors	Frequency	No.	HR	P	ORs (95% CIs)
Sex					
Male	28 (93.3)	13 (46.4)	1.75	0.598	0.22-13.99
Female	2 (6.7)	2 (100)			
Age	13 (43.3)	7 (53.8)			
< 60			0.933	0.873	0.34-2.18
≥ 60	17 (56.6)	8 (47.1)			

Multiplicity					
Simple	19 (63.3)	11 (57.9)	4.632	0.148	0.58-37.03
Multiple	11 (36.7)	4 (36.4)			
Tumor size					
<3 cm	14 (46.7)	6 (42.9)	1.094	0.894	0.29-4.07
≥ 3 cm	16 (53.3)	9 (56.3)			
Stage					
pTa	14 (46.7)	7 (50)	1.429	0.595	0.38-5.32
pT1	16 (53.3)	8 (50)			
Low	23 (76.7)	13 (56.5)	2.435	0.401	0.30-19.46
High	7 (23.3)	2 (28.6)			
ICAM-1 expression					
Low	19 (63.3)	4 (21)	0.024	0.194	0.0-6.70
High	11 (36.6)	11 (100)			
Abbreviations: HR: Hazard Ratio; 95%CI: Confidence Interval; p<0.05 is significant.					

Table 2: Univariate Cox regression analysis to predict time to recurrence following end of treatment: data are expressed as number and percentage of patients

Variable	P	HR	95% CI
Sex (F vs. M)	0.944	1.198	0.281-2.721
Age (<60 vs ≥ 60)	0.854	0.902	0.503-2.013
Grade(low vs. high)	0.414	1.005	0.603-1.490
Stage (T1 vs. Ta)	0.615	0.691	0.176-2.309
Tumor size(<3 vs ≥ 3)	0.737	0.852	0.257-2.785
Multiplicity	0.944	1.128	0.455-2.831
ICAM-1 (low vs. high)	0.939	1.026	0.219-2.768
Abbreviations: HR: Hazard Ratio; 95% CI: Confidence Interval; p<0.05 is significant.			

Table 3: Multivariate analysis of disease recurrence in patient with NMIBC treated by BCG immunotherapy

Discussion

One of the major issues of the BCG immunotherapy in NMIBC is to identify patients who could have refractory response and undergo to tumor recurrence. Currently, there are no relevant tumor characteristics correlated with a recurrence potential of these neoplasms after immunotherapy. Intravesical instillation of BCG is a standard therapy for NMIBC but its mechanism is not completely understood. Both immunological mechanisms and/or direct effects on tumor cells have been proposed, the former being more extensively studied. The direct effect on tumor cells has been less investigated in

part because the immune therapy with BCG is administered after tumor resection. However, it is possible that a number of tumor cells remain in the bladder mucosa and thus the biological behavior of urothelial cancer cells could play a role in the global response to instilled BCG.

Intercellular adhesion molecule-1 (ICAM-1), a cell adhesion molecule with a key role in inflammation and immune surveillance, has been implicated in carcinogenesis by facilitating instability of the tumor environment [10,11]. Several studies have demonstrated the role of ICAM-1 in a series of cancers, including gastric cancer, lung cancer, colorectal cancer, breast cancer, and prostate cancer. Some reports have demonstrated that, ICAM-1 is overexpressed in gastric cancer tissues, and this could be related to the aggressive nature of the tumor, and has a poor prognostic effect on gastric cancer [12]. In other studies, ICAM-1 is significantly elevated in patients with breast cancer compared with controls, and its expression is associated with a more aggressive tumor phenotype [13,14].

In relation to bladder carcinoma, until now, no study has found an association between ICAM-1 expression and the response of NMIBC to BCG therapy. Therefore, expression of ICAM-1 proteins by NMIBC cells seems to be a promising factor that must be assessed to evaluate its correlation with the recurrence rate. To the best of our knowledge, our current study is the first to investigate the relationship between ICAM-1 expression and the response to BCG therapy in patients suffering from NMIBC. In the present study, we described the prognostic value of ICAM-1 protein expression in NMIBC and its relationship with the response to BCG immunotherapy. Immunohistochemistry was used to analyze the correlation of ICAM-1 protein expression with clinical pathological factors and prognostic effect of 30 NMIBC patients. Hereby, we found that univariate Cox regression analysis of baseline characteristics and ICAM-1 expression showed that no significant association was found with BCG immunotherapy response. In the other hand, multivariate Cox regression analysis showed that ICAM-1 protein is not an independent factor of tumor response after BCG immunotherapy.

Our results are not in line with those of Anirban P et. al. who demonstrates that ICAM1 was a highly significant predictor of recurrence in urothelial carcinoma (UC) and the recursive partitioning (RP) analysis selected ICAM1 as joint determinant of recurrence. [15] In fact, they found that ICAM1 can predict clinical outcome in UC independent of conventional prognostic criteria and identify patients with operable UC who will experience recurrence despite undergoing definitive surgery alone. These patients would clearly benefit from additional therapy [15]. These results can be explained by the fact that in our study we included only non-muscle invasive bladder tumors while Anirban P et. al. have studied all types of urothelial tumors. Additionally, Fersching DM1 and al showed that ICAM-1 is valuable marker in breast cancer patients and show potential for early estimation of the efficiency of neoadjuvant chemotherapy. [16] In addition, previous study showed that increased levels of sICAM-1 have been reported in patients with several types of human malignancies. [17,18] Furthermore, the presence of sICAM-1 correlates with tumor progression and metastasis. [19-21] Recently, studies by Gho et al indicated that sICAM-1 may promote angiogenesis and stimulate tumor cell growth [8,22]. In patients who have NSCLC, increased levels of expression of sICAM-1 have been shown and its concentration is correlated with the clinical stage and tumor burden [23,24]. However, the role of sICAM-1 as surrogate marker of treatment efficacy and prognosis has not been investigated extensively.

On the other hand, Qian Q1 and al found that, Baseline sICAM-1 and sICAM-1 responses appeared to be reliable surrogate markers of chemotherapy efficacy and were prognostic factors in patients with advanced NSCLC [25].

Several limitations of this study should be considered. First, the sample size was small and follow-up was fairly short. Second, the clinical application of immunohistochemistry is limited by its reproducibility and reliability. Conversely, several strengths of the study should be noted. Our series is well selected, which provides valuable information and statistical significance.

Conclusion

This study demonstrates that ICAM-1 could not be a useful prognostic marker for BCG treatment in NMIBC. Therefore, additional studies using a larger scale of patients will be required to define the molecular pathways including ICAM-1 that effectively contribute to the response to the local immunotherapy.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Herr HW, Wartinger DD, Fair WR, Oettgen HF (1992) Bacillus Calmette-Guerin therapy for superficial bladder cancer: a 10-year followup. *J Urol* 147: 1020-1023.
- Sylvester RJ, van der MEIJDEN AP, Lamm DL (2002) Intravesical bacillus Calmette-Guerin reduces the risk of progression in patients with superficial bladder cancer: a meta-analysis of the published results of randomized clinical trials. *J Urol* 168: 1964-1970.
- Shahin O, Thalmann GN, Rentsch C, Mazzucchelli L, Studer UE (2003) A retrospective analysis of 153 patients treated with or without intravesical bacillus Calmette-Guerin for primary stage T1 grade 3 bladder cancer: recurrence, progression and survival. *J Urol* 169: 96-100.
- Lebret T, Bohin D, Kassardjian Z, Herve JM, Molinie V, et al. (2000) Recurrence, progression and success in stage Ta grade 3 bladder tumors treated with low dose bacillus Calmette-Guerin instillations. *J Urol* 163: 63-67.
- Rothlein R, Mainolfi EA, Czajkowski M, Marlin SD (1991) A form of circulating ICAM-1 in human serum. *J Immunol* 147: 3788-3793.
- Fonsatti E, Altomonte M, Coral S, Cattarossi I, Nicotra MR, et al. (1997) Tumour-derived interleukin 1alpha (IL-1alpha) up-regulates the release of soluble intercellular adhesion molecule-1 (sICAM-1) by endothelial cells. *Br J Cancer* 76: 1255-1261.
- Marino M, Scuderi F, Mazzarelli P, Mannella F, Provenzano C, et al. (2001) Constitutive and cytokine-induced expression of MHC and intercellular adhesion molecule-1 (ICAM-1) on human myoblasts. *J Neuroimmunol* 116: 94-101.
- Gho YS, Kleinman HK, Sosne G (1999) Angiogenic activity of human soluble intercellular adhesion molecule-1. *Cancer Res* 59: 5128-5132.
- Sauter G, Algaba F, Amin MB, et al. (2004) Non invasiveurothelial tumors. In: Elbe JN, Sauter G, Epstein JI, SesterhennIA,eds. *Pathology and Genetics: Tumors of the Urinary System andMale Genital Organs*. Lyon: IARC Press.
- Mousa SA (2008) Cell adhesion molecules: potential therapeutic & diagnostic implications. *MolBiotechnol* 38: 33-40.
- Manuel-Apolinar L, López-Romero R, Zarate A, Damasio L, Ruiz M, et al. (2013) Leptin mediated ObRb receptor increases expression of adhesion intercellular molecules and cyclooxygenase 2 on murine aorta tissue inducing endothelial dysfunction. *Int J ClinExp Med* 6: 192-196.
- Jung WC, Jang YJ, Kim JH, Park SS, Park SH, et al. (2012) Expression of intercellular adhesion molecule-1 and e-selectin in gastric cancer and their clinical significance. *J Gastric Cancer* 12: 140-148.
- O'Hanlon DM, Fitzsimons H, Lynch J, Tormey S, Malone C, et al. (2002) Soluble adhesion molecules (E-selectin, ICAM-1 and VCAM-1) in breast carcinoma. *Eur J Cancer* 38: 2252-2257.
- Schröder C, Witzel I, Müller V, Krenkel S, Wirtz RM, et al. (2011) Prognostic value of intercellular adhesion molecule (ICAM)-1 expression in breast cancer. *J Cancer Res ClinOncol* 137: 1193-1201.
- Mitra AP, Pagliarulo V, Yang D, Waldman FM, Datar RH, et al. (2009) Generation of a concise gene panel for outcome prediction in urinary bladder cancer. *J ClinOncol* 27: 3929-3937.
- Fersching DM, Nagel D, Siegele B, Salat C, Heinemann V, et al. (2012) Apoptosis-related biomarkers sFAS, MIF, ICAM-1 and PAI-1 in serum of breast cancer patients undergoing neoadjuvant chemotherapy. *Anticancer Res* 32: 2047-2058.
- Gearing AJ, Newman W (1993) Circulating adhesion molecules in disease. *Immunol Today* 14: 506-512.
- van de Stolpe A, van der Saag PT (1996) Intercellular adhesion molecule-1. *J Mol Med (Berl)* 74: 13-33.
- Alexiou D, Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, et al. (2001) Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. *Eur J Cancer* 37: 2392-2397.
- Viac J, Vincent C, Palacio S, Schmitt D, Claudy A (1996) Tumour necrosis factor (TNF) soluble receptors in malignant melanoma: correlation with soluble ICAM-1 levels. *Eur J Cancer* 32A: 447-449.
- Nakata B, Hori T, Sunami T, Ogawa Y, Yashiro M, et al. (2000) Clinical significance of serum soluble intercellular adhesion molecule 1 in gastric cancer. *Clin Cancer Res* 6: 1175-1179.
- Gho YS, Kim PN, Li HC, Elkin M, Kleinman HK (2001) Stimulation of tumor growth by human soluble intercellular adhesion molecule-1. *Cancer Res* 61: 4253-4257.
- Osaki T, Mitsudomi T, Yoshida Y, Oyama T, Ohgami A, et al. (1996) Increased levels of serum intercellular adhesion molecule-1 (ICAM-1) in patients with non-small cell lung cancer. *SurgOncol* 5: 107-113.
- Grothey A, Heistermann P, Philippou S, Voigtmann R (1998) Serum levels of soluble intercellular adhesion molecule-1 (ICAM-1, CD54) in patients with non-small-cell lung cancer: correlation with histological expression of ICAM-1 and tumour stage. *Br J Cancer* 77: 801-807.
- Qian Q, Zhan P, Yu L, Shi Y, Cheng J, et al. (2011) Baseline levels and decrease in serum soluble intercellular adhesion molecule1 during chemotherapy predict objective response and survival in patients who have advanced non-small-cell lung cancer. *Clin Lung Cancer* 12:131-137.