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The Significance of PDGF Expression in Serum of Colorectal Carcinoma Patients - Correlation with Dukes Clasification. Can PDGF Become a Potential Biomarker?

C. Braicu[1,2], O. Tudoran[1], L. Balacescu[1], C. Catana[3], E. Neagoe[4], I. Berindan-Neagoe[1,2,5], C. Ionescu[6,7]

[1]Department of Functional Genomics, "Ion Chiricuta" Oncology Institute, Cluj Napoca, Romania
[2]Research Center of Functional Genomics, Biomedicine and Translational Medicine
"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania
[3]Department of Biochemistry, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania
[4]Department of Surgery, The Oncology Institute "Ion Chiricuta", Cluj Napoca, Romania
[5]Department of Immunology, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania
[6]Department of Surgery, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania
[7]Surgery Clinic 1, County Hospital, Cluj Napoca Romania

Rezumat

Semnificația expresiei serice a PDGF pacienților cu cancer colorectal - corelații cu stadializarea Dukes. PDGF poate deveni un potențial biomarker?

Premise: Expresia la nivel seric a unor factori angiogenici a fost asociată cu diseminarea tumorală şi un prognostic nefavorabil în multe tipuri de cancer. Cu toate acestea, este încă neclar dacă aceste molecule implicate în angiogeneza tumorală pot fi folosite ca şi markeri moleculari independenți sau în corelație cu alți parametri pentru estimarea prognosticului în cazul pacienților cu carcinom colorectal (CRC).
Materiale şi metode: evaluarea expresiei proteice s-a realizat folosind kitul comercial *Angiogenesis fast quant protein array* pentru un număr de 28 de cazuri diagnosticate cu CRC şi 10 de cazuri de control.
Rezultate: a fost evidențiată subexprimarea PDGF-bb în cazul pacienților cu cancer colorectal, comparativ cu grupul de control. Astfel, PDGF-bb ar putea prezenta o funcție esențială în progresia şi validarea fenotipului malign şi a metastazării în cancerul colorectal.
Concluzii: Studiul nostru a indicat că expresia proteinei PDGF-bb ar putea fi un marker de prognostic independent sau în asociere cu alți parametri pentru pacienții CRC.

Cuvinte cheie: cancer colorectal, biomarkeri, proteine serice, PDGF-bb

Abstract

Background: The expression of serum angiogenic factors has been associated with tumor dissemination and poor prognosis in multiple cancer types. However, it is still unclear whether these angiogenic molecules can be used as an independent molecular marker or in correlation with other parameters for predicting the prognosis of colorectal carcinoma (CRC) patients.
Methods: Protein expression was evaluated in 28 CRC and 10 control cases using Angiogenesis Fast Quant technology.
Results: In this study, we found downregulation of PDGF-bb protein expression in the serum of patients with colorectal cancer compared with the control group. Thus, PDGF-bb might play an essential function in the progression of CRC.
Conclusions: Our study indicated that the PDGF-bb protein

Corresponding author: Ioana Berindan-Neagoe, MD
Research Center of Functional Genomics
Biomedicine and Translational Medicine
Iuliu Hatieganu University of Medicine
and Pharmacy, Cluj-Napoca, Romania
E-mail: ioana.neagoe@umfcluj.ro

expression might be an independent prognostic marker or in association with other parameters for CRC patients.

Key words: colorectal cancer, biomarkers, serum protein, PDGF-bb

Introduction

Colorectal cancer (CRC) is the third most common cancer in women and the fourth most common cancer in men worldwide (1). Related to the incidence of CRC Romania, CRC is the second most frequently diagnosed cancer in men after lung cancer and the third most frequently diagnosed cancer in women after breast and cervical cancer (2). Two staging procedures are routinely applied: Dukes's classification and the TNM (Tumour, Node, Metastasis) staging of tumours. Correct staging is fundamental since it furnishes the foundation selecting the therapeutic options, and supplies prognostic data for patients (3,4). Dukes's classification for CRC is relatively simple being well correlated with survival rate (5).

Since the mid-1930s, Dukes' staging system has been applied to colon cancer. In order to improve the survival rate of patients with CRC, an intensive research was done to develop non-invasive biomarkers for early diagnosis and prognosis. Although different panels of biomarkers were associated with Dukes staging system to improve current protocols in clinical practice, no definite biomarkers have been established (6). Consequently there is an imperative demand for the identification of non-invasive markers that can facilitate earlier detection of CRC. Therefore, numerous validation studies have been conducted to evaluate genetic, epigenetic or protein markers that can be quantified from the faecal or serum samples (7,8,9,10).

Tumor angiogenesis is a vital phase in the development, metastatic spread and recurrence of CRC (11,12,13). Tumor angiogenesis is a complex mechanism, which remains undeciphered. The balance among the endogenous angiogenic and anti-angiogenic factors may favour angiogenesis development and may have diagnostic and prognostic value. Metastasis of CRC is sustained by the effect of activation of proangiogenic factors (14,15).

Tumor angiogenesis plays a critical role in the growth and spread of cancer. CRC management has been improved due to new treatment approaches which combine chemotherapy with the use of monoclonal antibodies targeting the vascular endothelial growth factor (VEGF) pathway or EGFR (epidermal growth factor receptor) (16).

Despite the importance of this mechanism, no certified biomarkers of angiogenesis are accessible for routine clinical procedure. As a consequence, the search for novel angiogenic biomarkers and also their successful implementation in parallel with the recent advances in antiangiogenic therapy of cancer is a highly promising therapeutic approach in clinical oncology and a continuing challenge of translational research. There are also formerly no recognized biomarkers predicting disease staging for CRC. While k-ras oncogene mutations predict the response to EGFR monoclonal antibody therapy in patients with metastatic CRC (7), predictive or specific biomarkers are presently unavailable (13), because of these various types of molecules (8).

The aim of this study was to evaluate the clinical significance of serum angiogenic factors in relationship with Dukes classification, as novel molecules that may have prognostic value for invasive/metastatic capacity and prognosis of CRC. Identification of sensitive and specific colorectal cancer biomarkers opens up many perspectives for diagnostic and therapeutic exploitation. We evaluated the most commonly used serum angiogenic proteins such as: VEGF, PDFG (platelet-derived growth factor), FGF-b (Fibroblast Growth Factor-basic), KGF (Keratinocyte growth factor), angiogenin, angiopoietin-2, ICAM-1 (Intercellular Adhesion Molecule-1) and TIMP-1 (Tissue inhibitor of metalloproteinases-1).

Materials and Methods

Sample collection

Twenty-eight patients with colon adenocarcinoma and 10 healthy subjects were enrolled in this study. The blood samples were collected between 2006 and 2009 with approval of the Institutional Ethics Committee. All patients signed an informed consent. Blood samples were harvested in advance of any treatment or underwent biopsies. Serum samples were obtained by centrifugation at 3000 rpm for 5 min and stored at -80°C until further processing. Freezing and thawing of the serum among collection and processing was avoided.

Angiogenesis multiplex FAST Quant® protein array technology

By using Fast Quant® array technology (Whatman Schleicher&Schuell), we were able to simultaneously evaluate eight angiogenic molecules (VEGF, PDFG-bb, FGF-b, KGF, angiogenin, angiopoietin-2, ICAM-1, and TIMP-1) using specific monoclonal antibodies embedded on nitrocellulose membrane in triplicate to provide spot-to-spot and slide-to-slide reproducibility and specificity. The standard curve is assessed using fluorescent detection (Streptavidin-Cy5). The detection limits in the linear range are superior for most cytokines in the case of this protein microarray system than in the case of the classical ELISA. A 7-point extended wide selection standard curve was applied in order to increase the sensitivity for samples comprising very heterogeneous range of the analytes. The concentrations were expressed in pg/ml for all the analytes. After scanning with Axon GenePix® 4100A laser scanner (Molecular Devices), the images were processed with compatible imaging system software (ArrayVisionTM FAST® software). An average of the signal was used for each protein, after background subtraction; then the data were plotted as signal versus

concentration. The 7-point mass standard curve was used to quantify the concentrations of the proteins.

Statistical analysis

The statistical analysis for Fast Quant® array data were performed using the Statistical Package for Social Sciences (SPSS). The differences between three groups were assessed by Kruskal-Wallis test followed by Dunn's multiple comparison tests ($p < 0.05$ was considered significant).

Results

Serum from 28 CRC patients and 10 healthy controls with no obvious presence of gastrointestinal diseases at colonoscopy were selected for the evaluation of serum angiogenic profile (*Table 1*). From the selected CRC cases, 12 were classified as Dukes B, 10 cases were classified as Dukes C and 6 cases were classified as Dukes D (*Table 2*).

Among the eight molecules investigated (PDFG-bb, VEGF, FGF-b, angiogenin, KGF, TIMP-1, ICAM-1, and angiopoetin–2), only angiopoetin–2 and PDFG-bb could be quantified using the standard curve. The values of the other molecules (VEGF, angiogenin, TIMP-1, ICAM-1, FGF-b and KGF) lie outside the range of the standard curve. The protein expression level for PDGF-bb and angiopoietin-2 are presented in *Table 3*, *Fig. 1* and *Fig. 2*.

PDGF-bb median value in the control group was 7442.13 (pg/ml) with a range among 3800.82 - 12066.99 (pg/ml). For Dukes B group, PDGF-bb values were ranged 309.32 - 9457.96 (pg/ml), while for Dukes C+D group, the values were ranged 1205.08 - 7815.09 (pg/ml) (*Table 4*).

Comparison of protein levels in control, Dukes B and Dukes C+D groups was performed with nonparametric Kruskal-Wallis test. Statistically significant differences were observed only for PDGF-bb levels in Dukes B and Dukes C+D groups versus control group. The protein expression of angiopoietin-2 was not significantly altered in Dukes B and C+D groups compared with control (*Table 4*).

Discussions

In our study the serum levels of multiple angiogenesis-related proteins in both patients with CRC and controls were determined using FAST Quant protein arrays system. All the molecules were simultaneously measured using a 50 μl volume of sample serum. Since the diagnostic options for CRC are usually invasive, our approach is to find serum biomarkers that can be safely and repetitively used.

As was emphasized by a similar recent study (17) conducted by Krzystek-Korpacka, the necessity of developing alternative CRC screening and surveillance strategies to replace the invasive methods is required. Multiplexed analyses of cytokines were able to discriminate CRC patients from controls,

Table 1. *Group of study*

Patient ID	Age	Grade	TNM stage	Dukes	LV invasion
P01	65	G1	T2N1M0	Dukes C	-
P02	38	G3	T3N2M0	Dukes C	L1V1
P03	56	G2	T4N2M1	Dukes D	L1V1
P04	51	G3	T4N0Mx	Dukes C	L0V0
P05	48	G1	T2N0Mx	Dukes B	L1V1
P06	66	G2	T3N1M0	Dukes C	L1V0
P07	77	G2	T4N2Mx	Dukes D	L1V0
P09	72	G1	T3N0M0	Dukes B	L1V0
P10	79	G2	T3N0M0	Dukes B	L1V0
P13	59	G2	T3N0M0	Dukes B	L0V0
P14	70	G2	T2N0M0	Dukes B	L1V0
P15	70	G3	T3N2M1	Dukes D	L1V0
P16	71	G2	T3N0M0	Dukes B	L1V0
P20	57	G2	T2N1Mx	Dukes C	L1V1
P21	55	-	T3N0M1	Dukes D	L0V0
P22	75	G2	T3N1M0	Dukes C	L1V0
P23	81	G2	T2N1M0	Dukes C	L1V1
P24	66	G2	T2N0M0	Dukes B	-
P27	72	G2	T3N1M0	Dukes C	L0V0
P28	40	G2	T2N0M0	Dukes B	L0V0
P29	65	G2	T3N1M1	Dukes D	L1V0
P31	68	G2	T3N1M0	Dukes C	-
P33	75	G2	T3N2M1	Dukes D	-
P35	64	-	T3N0M0	Dukes B	L0V0
P36	80	G2	T2N2M0	Dukes C	L1V0
P38	47	G2	T3N0M0	Dukes B	L1V0
P39	76	G3	T3N0M0	Dukes B	L0V0
P40	72	G2	T3N0M0	Dukes B	L1V0

Table 2. *Patient characteristics*

	No. of patients	%	Dukes B	Dukes C	Dukes D
Study group	28	100	12	10	6
Age					
< 50	4	14.28	3	1	-
50-60	5	17.86	1	2	2
60-70	6	21.43	2	3	1
≥ 70	13	46.43	6	4	3
Sex					
M	20	71.43	9	6	5
F	8	28.57	3	4	1

Table 3. *Serum concentration of Angiopoietin-2 and PDGF-bb in studied groups*

	Patient ID	Angiopoietin-2 concentration (pg/ml)	PDGF-bb concentration (pg/ml)
Control group			
	C1	8012.24	8364.48
	C2	13952.34	8557.69
	C3	13476.25	4357.10
	C4	11963.32	5164.11
	C5	14422.14	3800.83
	C6	9832.30	6132.11
	C7	14039.19	9855.79
	C8	5002.64	7787.67
	C9	5087.11	12066.99
	C10	7906.47	7096.58
Dukes B			
	P05	13537.61	3657.82
	P09	9159.40	309.32
	P10	10074.90	4978.45
	P13	16285.51	9457.96
	P14	13824.99	2992.83
	P16	11213.35	3358.41
	P24	9659.07	3772.69
	P28	10248.18	4177.05
	P35	10343.15	4387.53
	P38	6507.40	4452.14
	P39	25108.26	3105.51
	P40	16078.17	8414.04
Dukes C+D			
	P01	12805.86	7815.10
	P02	19167.39	6545.78
	P03	11610.78	4082.87
	P04	19299.00	5611.86
	P06	9298.37	under
	P07	8497.02	5907.75
	P15	11851.00	3063.70
	P20	3262.21	1205.08
	P21	11418.40	4606.77
	P22	8010.51	1293.87
	P23	15876.35	1637.09
	P27	6786.09	1837.17
	P29	19390.31	5747.60
	P31	17247.63	3146.61
	P33	13145.27	5290.46
	P36	7296.74	5220.33

adenomas, or inflammatory bowel disease patients with relatively good accuracy (9).

To improve diagnostic and treatment efficacy, it is necessary to develop screening serum testing with minimal invasiveness and cost-effectiveness. In cohort studies, differential expression of inflammatory serum biomarkers in healthy controls compared to individuals with different stages of CRC was detected (13,18).

The survival and growth of CRC and consequently their metastases are related to the balance of endogenous angiogenic factors so that the outcome favours amplified angiogenesis (6), justifying the necessity of the serum determination and the correlation with Dukes staging system. Taking into account the fact that tumor development, growth, and metastasis are tightly controlled by this "endogenous angiogenic balance", measurement of serum factors and the correlation between them and the Dukes staging system is becoming a necessity as well as the idea of personalized therapy in CRC (19,20).

Levels of the main angiogenic factors, such as vascular endothelial growth factor-A (VEGF-A) and angiopoietin-2

Table 4. *Comparison of Angiopoietin-2 and PDGF-bb levels in healthy subjects vs. colon cancer with Dukes classifications B vs. colon cancer with Dukes classifications C and D (Kruskal Wallis test. *: p<0.05)*

	Angiopoietin-2 (pg/ml)				PDGF-bb (pg/ml)			
	median	**range**	**p**	**sig.**	**median**	**range**	**P**	**sig.**
Control	10897.81	5002.64-14422.14	0.68	ns	7442.13	3800.82-12066.99	0.0145	*
Dukes B	10778.25	6507.39-25108.26			3974.87	309.32-9457.96		
Dukes C+D	11730.89	3262.21-19390.31			4606.77	1205.08-7815.09		

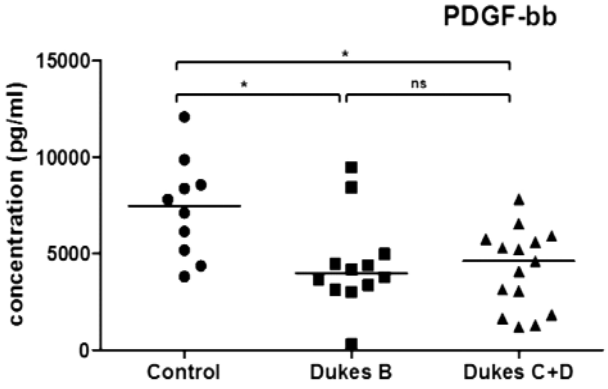

Figure 1. *Serum concentration for PDGF-bb in control, Dukes B and Dukes C+D groups. The differences between groups were assessed by Kruskal-Wallis test followed by Dunn's multiple comparison test (ns-not significant, *p<0.05; **p<0.01; ***p<0.001). The horizontal line represents the median*

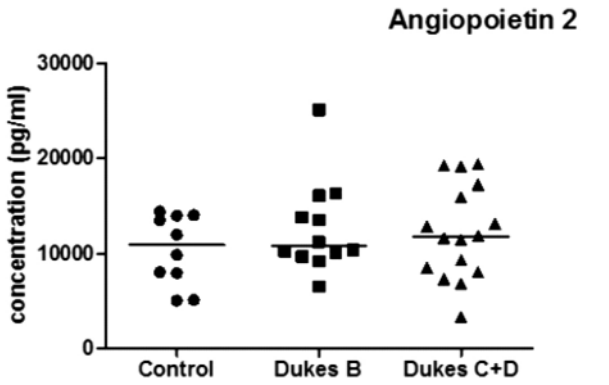

Figure 2. *Serum concentration of Angiopoietin 2 in control, Dukes B and Dukes C+D groups. The differences between groups were assessed by Kruskal-Wallis test followed by Dunn's multiple comparison test (ns-not significant, *p<0.05; **p<0.01; ***p<0.001). The horizontal line represents the median. No statistical significant differences were observed*

(Ang-2), are correlated with tumour progression and patient outcome in CRC (21,22). VEGF expression at the deepest invasive site of tumour is the most statistically significant prognostic indicator in advanced colorectal carcinoma (6). It was expected, that together with PDGF-bb, it would be overexpressed in the patient serum (6). In our study, we observed under-expression of PDGF-bb in patients serum compared to healthy subjects.

Studies have revealed that the plasma levels of all the angiogenesis-related molecules were markedly increased in colorectal cancer patients compared with the levels of those with pancreatic cancer, pancreatitis, as well as benign hepato-biliary diseases. (23)

The VEGF monoclonal antibody bevacizumab has been shown to provide a significant clinical benefit in colorectal cancer when added to chemotherapy. In addition, this factor has been already widely used for standard therapy (11). In accordance, a statistically significant increase in PDGF and bFGF level was noted in Dukes B CRC patients compared with Dukes C patients. Concerning the VEGF level, an increase regardless of tumor stage was observed (24).

On the one hand, PDGF-bb was proved to be involved in human malignancies. Its overexpression has been reported in some human tumors. However, its expression in colorectal cancer has not been studied. Therefore, we investigated the clinicopathological correlations and also the biological significance of PDGF-bb gene expression in human colorectal cancer (25). Other clinical studies confirmed that PDGF-bb expression could be a new prognostic biomarker in colorectal cancer patients with colorectal cancer (11). In this context, our findings about the PDGF-bb, which was overexpressed in the patients' serum, are beneficial in the case of colorectal cancer therapy.

Moreover, platelet-derived growth factor-bb levels were greater in patients with colorectal cancer compared with patients with adenoma and according to the increasing disease severity. Unfortunately, the modest differences between the two groups did not permit accurate stage determination (26).

On the other hand, angiopoietin-2 represents an important inhibitory molecule of the Tie-2 receptor that is stowed in the Weibel–Palade bodies from the endothelial cells (27). Angiopoietin-2 can disturb the integrity of the blood vessel barrier, consequently counterbalancing vascular normalisation (28,29). Based on this fact, we anticipated angiopoietin-2 to be mainly expressed in the stromal part of CRC and assumed to have a high expression of angiopoietin-2 in CRC patients. In spite of the fact that serum angiopoietin-2 was presented as a candidate biomarker for outcome of patients with metastatic CRC (8), we did not observe significant differences between the CRC groups and controls. Taking into account that

angiopoietin-2 is originated from the stromal compartment of CRC tissues, serum angiopoietin-2 levels were considerably overexpressed in the case of patients with metastatic CRC compared with healthy subjects (8-30). In the case of patients receiving bevacizumab treatment, angiopoietin-2 downregulation was correlated with an increased response to treatment and a better survival rate.

Conclusions

In conclusion, our results indicated an association between PDGF-bb and Dukes stages. The combination of PDGF-bb and the Dukes staging system could be a promising factor for cancer management and could also facilitate the early intervention in cancer chemotherapy in order to benefit from the available treatments.

References

1. Fukushima T, Akagi Y, Murakami H, Ushijima M, Ogata Y, Shirouzu K, et al. Estimation of the number of metastatic lymph nodes in dukes' C colorectal cancer. Anticancer Res. 2013;33(7):2949-55.
2. http://globocan.iarc.fr/factsheet.asp#MEN
3. Verrill C, Carr NJ, Wilkinson-Smith E, Seel EH. Histopathological assessment of lymph nodes in colorectal carcinoma: does triple levelling detect significantly more metastases? J Clin Pathol. 2004;57(11):1165-7.
4. Nastase A, Pâslaru L, Niculescu AM, Ionescu M, Dumitraşcu T, Herlea V, et al. Prognostic and predictive potential molecular biomarkers in colon cancer. Chirurgia (Bucur). 2011;106(2):177-85.
5. Adachi Y, Mori M, Maehara Y, Sugimachi K, Dukes's classification: a valid prognostic indicator for gastric cancer. Gut. 1994;35(10):1368-71.
6. Tiernan JP, Perry SL, Verghese ET, West NP, Yeluri S, Jayne DG, et al. Hughes Carcinoembryonic antigen is the preferred biomarker for in vivo colorectal cancer targeting. Br J Cancer. 2013;108(3):662-7.
7. Bolocan A, Ion D, Ciocan DN, Paduraru DN. Prognostic and predictivefactors in colorectal cancer. Chirurgia (Bucur). 2012; 107(5):555-63.
8. Helgason HH, Engwegen JY, Zapatka M, Vincent A, Cats A, Boot H, et al. Identification of serum proteins as prognostic and predictive markers of colorectal cancer using surface enhanced laser desorption ionization-time of flight mass spectrometry. Oncol Rep. 2010;24(1):57-64.
9. Helgason HH, Engwegen JY, Zapatka M, Cats A, Boot H, Beijnen JH, et al. Serum proteomics and disease-specific biomarkers of patients with advanced gastric cancer. Oncol Lett. 2010;1(2):327-333.
10. Olariu A, Bartoş A, Bartoş D, Neciu C, Mocan L, Zaharie F, et al. Evaluation of prognostic factors for colon cancer. Chirurgia (Bucur). 2012;107(2):174-9. Romanian
11. Rmali KA, Puntis MC, Jiang WG. Tumour-associated angiogenesis in human colorectal cancer. Colorectal Dis. 2007;9(1): 3-14.
12. Goi T, Fujioka M, Satoh Y, Tabata S, Koneri K, Nagano H, et al. Angiogenesis and tumor proliferation/metastasis of human colorectal cancer cell line SW620 transfected with endocrine glands-derived-vascular endothelial growth factor, as a new angiogenic factor. Cancer Res. 2004;64(6):1906-10.
13. Belur LR, Podetz-Pedersen KM, Sorenson BS, Hsu AH, Parker JB, Carlson CS, et al. Inhibition of angiogenesis and suppression of colorectal cancer metastatic to the liver using the Sleeping Beauty Transposon System. Mol Cancer. 2011;10(1):14.
14. Rmali KA, Puntis MC, Jiang WG. Tumour-associated angiogenesis in human colorectal cancer. Colorectal Dis. 2007;9(1): 3-14.
15. Miron N, Susman S, Balacescu O, Buiga R, Berindan-Neagoe I, Cristea V, et al. Novel cellular and molecular approaches to stratification and treatment of colorectal cancer. J Gastrointestin Liver Dis. 2012;21(4):413-21.
16. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008;359(17):1757-65.
17. Krzystek-Korpacka M, Diakowska D, Kapturkiewicz B, Bebenek M, Gamian A. Profiles of circulating inflammatory cytokines in colorectal cancer (CRC), high cancer risk conditions, and health are distinct. Possible implications for CRC screening and surveillance. Cancer Lett. 2013;337(1):107-14.
18. Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010;138(6):2101-14.
19. Kulendran M, Stebbing JF, Marks CG, Rockall TA. Predictive and prognostic factors in colorectal cancer: a personalized approach. Cancers (Basel). 2011;3(2):1622-38.
20. Shibayama M, Maak M, Nitsche U, Gotoh K, Rosenberg R, Janssen KP. Prediction of metastasis and recurrence of colorectal cancer based on gene expression analysis: ready for the clinic? Cancers (Basel). 2011;3(3):2858-69.
21. Sawada T, Yashiro M, Sentani K, Oue N, Yasui W, Miyazaki K, et al. New molecular staging with G-factors (VEGF-C and Reg IV) by supplementing TNM classification in colorectal cancers. Oncol Rep. 2013;30(6):2609-16.
22. Wang R, Xie J, Chen H, Ren T, Zhang Y. Expression of angiopoietin-2 and vascular endothelial growth factor in human colon cancer. Nan Fang Yi Ke Da Xue Xue Bao. 2013;33(8):1236-9. Chinese
23. Sakamoto H, Kimura H, Sekijima M, Matsumoto K, Arao T, Chikugo T, et al. Plasma concentrations of angiogenesis-related molecules in patients with pancreatic cancer. Jpn J Clin Oncol. 2012;42(2):105-12.
24. Ionescu C, Berindan-Neagoe I, Burz C, Balacescu O, Balacescu L, Tudoran O, et al. The clinical implications of platelet derived growth factor B, vascular endothelial growth factor and basic fibroblast growth factor in colorectal cancer. J BUON. 2011; 16(2):274-6.
25. Nakamura Y, Tanaka F, Yoshikawa Y, Mimori K, Inoue H, Yanaga K, et al. PDGF-BB is a novel prognostic factor in colorectal cancer. Ann Surg Oncol. 2008;15(8):2129-36.
26. Belizon A, Balik E, Horst PK, Shantha-Kumara HM, Nasar A, Whelan RL. Platelet-derived growth factor (subtype BB) is elevated in patients with colorectal carcinoma. Dis Colon Rectum. 2009;52(6):1166-71.
27. Fiedler U, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, et al. The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. Blood. 2004;103(11):4150-6.
28. Falcón BL, Hashizume H, Koumoutsakos P, Chou J, Bready JV, Coxon A, et al. Contrasting actions of selective inhibitors of angiopoietin-1 and angiopoietin-2 on the normalization of tumor blood vessels. Am J Pathol. 2009;175(5):2159-70.
29. Reiss Y, Knedla A, Tal AO, Schmidt MH, Jugold M, Kiessling F, et al. Switching of vascular phenotypes within a murine breast cancer model induced by angiopoietin-2. J Pathol. 2009;217(4): 571-80.
30. Goede V, Coutelle O, Neuneier J, Reinacher-Schick A, Schnell R, Koslowsky TC, et al. Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. Br J Cancer. 2010; 103(9):1407-14.