RESEARCH ARTICLE

Hassan Ali Al-Saadi

Immunohistochemical study of IL-17, perforin and CD68 in colorectal cancer

Authors' address:
Department of Clinical Laboratory Sciences, Pharmacy College, Kerbala University, Kerbala, Iraq.

Correspondence:
Dr. Hassan Ali Al-Saadi
Department of Clinical Laboratory Sciences, Pharmacy College, Kerbala University, Kerbala, Iraq.
e-mail: drhassan54@gmail.com

Article info:
Received: 29 August 2014
Accepted: 15 September 2014

ABSTRACT

The tumor development in colorectal cancer is one of the most investigated tumorigenesis in the world. This study aims to analyze the impact of presence of IL-17, perforin and CD68 on colorectal cancer. During the period October 1st 2013 – March 1st 2014, samples from fifty patients with colon cancer (14 female and 36 male, 7-75 years old) from Al-Hussain Hospital City (Kerbala, Iraq), Hospital Medical City (Baghdad, Iraq) and Teaching Oncology Hospital, Baghdad Medical City (Baghdad, Iraq) were taken and analyzed. Immunohistochemical analyses of IL-17, perforin and CD68 of colorectal biopsies were performed by using Dako's Service (Denmark). The levels of IL-17 were significantly ($p<0.01$) higher in females (age group 41-75), and Duke C. Perforin was found around the crypts of the mucosa and submucosa. Their levels were significantly ($p<0.01$) higher in males (age group 41-75) with significance ($p<0.01$) in Duke B. CD68 was present around the crypts, but results showed no significant difference ($p>0.05$) between the genders, whereas there was a highly significant ($p<0.01$) increase in age group 41-75 when compared with the others groups. Also, the results revealed a highly significant raise ($p<0.01$) in Duke C when compared with the TNM groups and highly significant ($p<0.01$) correlation among study parameters. These results demonstrated the critical role of IL-17, perforin and CD68 highlighting tumorigenesis and inflammation as pro-inflammatory factors in colorectal cancer.

Key words: IL-17, perforin, CD68, colorectal cancer

Introduction

The tumor development in colorectal cancer (CRC) is one of the most studied tumorigenesis in the world. CRC is characterized by a complex interaction between genetic alterations, environmental carcinogens, and the host immune system, ultimately resulting in the uncontrolled growth of transformed cells (Terzić et al., 2010). Mechanisms that underlying the linkage between inflammation and cancer are incompletely understood (Straus, 2013). Interleukin 17 (IL-17) is a proinflammatory cytokine produced by activated T cells and other immune cells (Starnes et al., 2001; Kawaguchi et al., 2001). It is newly described cytokine, which plays an important role in the innate and adaptive immune systems. IL-17 is produced by a lineage of $CD4^+$ T helper cells named Th-17 cells. Th-17 induces release of proinflammatory and neutrophil-mobilizing cytokines, mostly through IL-17 secretion in several chronic pathologies such as inflammatory and autoimmune diseases (McGovern et al., 2009).

Cytotoxic granules of perforins are intracellular molecules present in the lymphocytes and NK cells. Perforin is required for ability of granzyme B to promote apoptosis in the target cells (Stepp et al., 1999; Chowdhury & Lieberman, 2008). NK cells express highly levels of perforin that is closely associated with cytotoxicity of NK cells (Bhat & Watzl, 2007). Confirmed evidence from studies in mouse models and human cancer patients suggests that tumorigenesis and progression is not only dominated by genetic alterations intrinsic to the tumor cells, but also by epigenetic and tumor microenvironmental factors. It has become clear that the host immune system is a microenvironmental factor that altered tumor development (Kaplan et al., 1998). Macrophages are usually the most copious immune population in the tumor microenvironment

DOI: 10.69085/jbb20143261

RESEARCH ARTICLE

(Allavena et al., 2008; Erreni et al., 2011). The role of macrophages in colorectal cancer tumorogenesis is complex, because they can both promote and prevent tumor development (Gulubova et al., 2013). The aim of this study was to analyze the impact of presence of IL-17, perforin and CD68 on colorectal cancer.

Patients and Methods

Patients

Patients with colon cancer (14 female and 36 male, 7-75 years old) were selected from Al-Hussain Hospital City (Kerbala, Iraq), Hospital Medical City (Baghdad, Iraq) and Teaching Oncology Hospital Baghdad Medical City (Baghdad, Iraq). Tumors were staged according to the TNM criteria (Greene & Sobin, 2008). Known tumor characteristics included differentiation grade. Samples were taken during the period October 1st 2013 – March 1st 2014.

Immunohistochemistry techniques (Dako, Denmark): Novolink polymer detection systems

FFPE (formalin-fixed paraffin-embedded) tissue sections of 5 µm thickness were prepared on aminopropylethoxysilane (APES)-coated slides and dried at 37°C. Sections were deparaffinized by two changes of xylene for 4 min each, rehydrated in graded ethanol and washed in tap water. Antigen was unmasked in tris-EDTA buffer pH 9 or sodium citrate pH 6 and microwaved for 25 min at 800 or 600 watt, respectively. After cooling for 15 min, biopsies were washed in deionized water. One-two drops of peroxidase blocking solution were added for 5 min, then washed in PBS for 2x5 min at room temperature and then washed again twice with PBS. After adding one-two drops of protein blocking solution for 5 min, samples were washed in PBS (2x5 min). 50 µl of monoclonal Abs (1 µl/100 ml PBS; anti-IL-17, anti-perforin and anti-CD68), were added to each section and incubated for 1 hour at room temperature. After washing with PBS, one drop post primary block solution was added for 30 min, and then washed twice with PBS. One drop of Polymer AB secondary Ab was added for 30 min and washed twice again with PBS. DAB buffer (50 µl) was added for each section (1 µl DAB / 50 µl DAB buffer). Then sections were incubated for 8 min and washed with PBS. One drop of hematoxyline was added for 1 - 1.5 min, and then washed with PBS and tap water. Samples were mounted on the glass by adding one drop of oil-cover and covered with a coverslip.

Microscopically, the samples were calculated with '0' equaling no positive cells, '1' minimal (5%), '2' moderate (5-10%), '3-4' abundant (>10%) quantities of positive cells (Hussain et al., 1994).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Student *t*-test, ANOVA and Pearson correlation were used to analyze results by using SPSS version 22. P-values ≤ 0.05 were considered as significant.

Results

Fifty colorectal cancer biopsies specimens from patients were evaluated. Thirty six of 50 patients were male (72%) and fourteen (28%) were female. Median age at diagnosed stage was 59 in a range of 7-75 years. All patients had stages A, B and C according to Duke score (Table 1).

Table 1. *Characteristic features of patients.*

Characteristic	**N**	**%**
Number of patients	50	100
Sex		
Male	36	72
Female	14	28
Age (years)		
Median	59	
Range	7-75	
TNM		
Duke A	5	10
Duke B	28	56
Duke C	17	34

Location and numbers of IL-17, perforin and CD68 positive cells within the tumor tissues were determined. IL-17 was found to be present mainly in the mucosa around the crypts and few were detected in the submucosa. Their levels were significantly ($p<0.01$) higher in females (age group 41-75 years) and Duke C. Perforin was found around the crypts of the mucosa and submucosa. Their levels were significantly ($p<0.01$) higher in males (age group 41-75) with significance ($p<0.01$) in Duke B. CD 68 was found around the crypts, but the results showed no significant ($p>0.05$) difference. There was a highly significant ($p<0.01$) increase in age group 41-75 years compared with the others groups. Also, results revealed

DOI: 10.69085/jbb20143261

RESEARCH ARTICLE

a highly significant ($p<0.01$) raise in Duke C compared with the TNM groups (Table 2, Figure 1). Our results showed highly significant ($p<0.01$) correlation among studied parameters (Table 3).

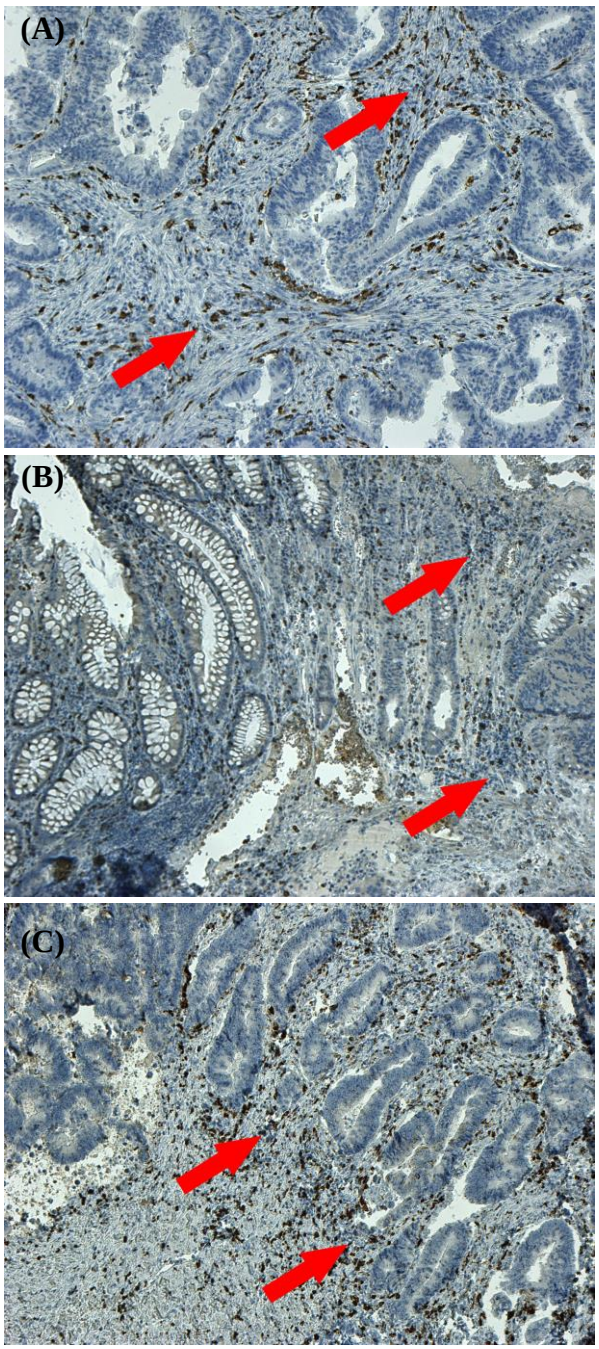

Figure 1. *Immunohistochemical staining of colorectal biopsies.*
(A). IL-17, Duke A:T2N0Mx; (B). perforin, Duke B:T3N0Mx; (C). CD68, Duke B:T3N0Mx. Original magnification 400x.

Discussion

Our results showed the importance of IL-17, perforin and CD68 in colorectal cancer. Previous studies were suggested that IL-17, a proinflammatory cytokine produced by Th17 cells, playing a binary role in the antitumor immunity. So, it promotes an antitumor cytotoxic T cell response leading to tumor regression. The antitumor effects of IL-17 are thus functions of the IL-17-induced inflammatory mediators, and these mediators regulate IL-17 production, all operating in tandem (Murugaiyan & Saha, 2009). The exact mechanisms implied the generation of IL-17$^+$ Treg cells remain unclear (Li & Boussiotis, 2013). Levels of IL-17 in mice were increased in the colon tissues, while IL-17A plays important roles in cancer development. The function of IL-17F in tumorigenesis has not been valued yet. These results indicated that IL-17F plays an inhibitory role in colon tumorigenesis *in vivo* (Tong et al., 2012). It remains unclear what sets of granzymes and perforin are expressed by the various immune cells and how cytolytic enzyme content relates to the cellular differentiation (Chattopadhyay et al., 2009). Perforin and granzymes released by CD8$^+$ CTLs will be functionally activated to destroy infected and tumor cells (Naito et al., 1998). Cytotoxic T cells and natural killer cells are able to solicited apoptosis of harmful target cells in a perforin-dependent manner. Perforin is contributed in both the regulation of activity of mucosal inflammation and the antitumor immune response in colitis-associated cancer (Waldner et al., 2010). The role of macrophages is complex, because they can both prevent and promote tumor development (Gulubova et al., 2013). Macrophages are present in the stroma of colon carcinoma and in regional metastasis free lymph nodes. The number of tumor macrophages varied between cases and the different tumor markers (Faber et al., 2012). The role of tumor-associated macrophages and IL-17 is still unclear (Erreni et al., 2011).

Conclusion

Our results showed the importance of IL-17, perforin and CD68 in colorectal cancer immune response. They supposed that tumor cell mediators have an effect on macrophages leading to secretion of IL-17. The immunoregulatory functions, which induced tumor apoptosis by perforin sharing, is in conformity with genetic evolution, such genetic link between IL-17, perforin and CD68. Thus, cancers may allow in the future a new vistas conquering testing to better evaluate the risk of cancer and the therapeutic response.

DOI: 10.69085/jbb20143261

RESEARCH ARTICLE

Table 2. *Values of IL-17, perforin and CD68 dispensed according to gender, age and TNM. Data are presented as density (cells/mm^3).*

Characteristic	IL-17		Perforin		CD68	
	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Gender						
Male (36)	1.97 ± 0.44	< 0.001	2.33 ± 0.67	< 0.001	2.8 ± 0.37	0.8
Female (14)	2.85 ± 0.36		1.28 ± 0.46		2.85 ± 0.36	
Age						
(7-20) 3	1 ± 0.001		1 ± 0.01		2 ± 0.01	
(21-40) 5	1.8 ± 0.4	< 0.001	1.8 ± 0.4	0.035	2 ± 0.01	< 0.001
(41-75) 42	2.35 ± 0.48		2.1 ± 0.7		3 ± 0.01	
TNM						
Duke A (5)	1.4 ± 0.54		1.4 ± 0.54		2 ± 0.01	
Duke B (28)	1.96 ± 0.18	< 0.001	2.5 ± 0.57	< 0.001	2.8 ± 0.31	< 0.001
Duke C (17)	2.88 ± 0.33		1.41 ± 0.5		3 ± 0.01	

Table 3. *Correlation between the parameters.*

Parameters	r	p-value
IL-17 vs. CD 68	0.6	< 0.001
IL-17 vs. perforin	-0.3	0.01
Perforin vs. CD 68	0.3	0.03

Acknowledgement

The author is greatly appreciated the support of the medical staff in Education Hospital Medical City Baghdad and Teaching Oncology Hospital, Baghdad Medical City (Baghdad, Iraq), Dr. Ali R. (lab manager), Dr. Nazar J. and Dr. Haider J. (Department of Pathology), Dr. Rasha, pharmacist Shawad Baqer (Department of Immunology, Al-Hussien Hospital, Kerbala, Iraq).

References

Allavena P, Sica A, Solinas G, Porta C, Mantovani A. 2008. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit. Rev. Oncol. Hematol., 66: 1–9.

Bhat R, Watzl C. 2007. Serial killing of tumor cells by human natural killer cells–enhancement by therapeutic antibodies. PLoS One, 2(3): e326.

Chattopadhyay PK, Betts MR, Price DA, Gostick E, Horton H, Roederer M, De Rosa SC. 2009. The cytolytic enzymes granyzme A, granzyme B, and perforin: expression patterns, cell distribution, and their relationship to cell maturity and bright CD57 expression. J. Leukoc. Biol., 85(1): 88-97.

Chowdhury D, Lieberman J. 2008. Death by a thousand cuts: granzyme pathways of programmed cell death. Annu. Rev. Immunol., 26: 389–420.

Erreni M, Mantovani A, Allavena P. 2011. Tumor-associated macrophages (TAM) and inflammation in colorectal cancer. Cancer Microenvironment, 4: 141–154.

Faber TJE, Japink D, Leers MPG, Sosef MN, Meyenfeldt MF, Nap M. 2012. Activated macrophages containing tumor marker in colon carcinoma: immunohistochemical proof of a concept. Tumor Biol., 33(2): 435-441.

Greene FL, Sobin LH .2008. The staging of cancer: a retrospective and prospective appraisal. CA Cancer J. Clin., 58(3): 180–190.

Gulubova M, Ananiev J, Yovchev Y, Julianov A, Karashmalakov A, Vlaykova T. 2013. The density of macrophages in colorectal cancer is inversely correlated to TGF-b1 expression and patients' survival. J. Mol. Hist., 44: 679–692.

Hussain MJ, Mustafa A, Gallati H, Mowat AP, Mieli-Vergani G, Vergani D. 1994. Cellular expression of tumour necrosis factor-alpha and interferon-gamma in the liver biopsies of children with chronic liver disease. J. Hepatol., 21: 816-821.

Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, Schreiber RD. 1998. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc. Natl. Acad. Sci. USA, 95: 7556-7561.

Kawaguchi M, Onuchic LF, Li XD, Essayan DM, Schroeder J, Xiao HQ, Liu MC, Krishnaswamy G, Germino G, Huang SK. 2001. Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. J. Immunol., 167: 4430–4435.

Li L, Boussiotis VA. 2013. The role of IL-17-producing Foxp3+ CD4+ T cells in inflammatory bowel disease and colon cancer. Clinical Immunology, 148: 246–253.

DOI: 10.69085/jbb20143261

RESEARCH ARTICLE

McGovern DP, Rotter JI, Mei L, Haritunians T, Landers C, Derkowski C, Dutridge D, Dubinsky M, Ippoliti A, Vasiliauskas E, Mengesha E, King L, Pressman S, Targan SR, Taylor KD. 2009. Genetic epistasis of IL23/IL17 pathway genes in Crohn's disease. Inflamm. Bowel Dis., 15: 883–889.

Murugaiyan G, Saha B. 2009. Protumor vs antitumor functions of IL-17. J. Immunol., 183: 4169-4175.

Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H, and Ohtani H. 1998. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res, 58: 3491-3494.

Starnes T, Robertson MJ, Sledge G, Kelich S, Nakshatri H, Broxmeyer HE, Hromas R. 2001. Cutting edge: IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. J. Immunol., 167: 4137–4140.

Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, Henter JI, Bennett M, Fischer A, de Saint Basile G, Kumar V. 1999. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science, 286: 1957–1959.

Straus DS. 2013. TNF-α and IL-17 cooperatively stimulate glucose metabolism and growth factor production in human colorectal cancer cells. Molecular Cancer, 12: 78-91.

Terzić J, Grivennikov S, Karin E, Karin M. 2010. Inflammation and colon cancer. Gastroentrology, 138: 2101-2114.

Tong Z , Yang XO, Yan H, Liu W, Niu X, Shi Y, Fang W, Xiong B, Wan Y, Dong C. 2012. A Protective role by interleukin-17F in colon tumorigenesis, PLoS One, 7(4): e34959.

Waldner MJ, Wirtz S, Becker C, Seidel D, Tubbe I, Cappel K, Hähnel PS, Galle PR, Schuler M, Neurath MF. 2010. Perforin deficiency attenuates inflammation and tumor growth in colitis-associated cancer. Inflamm Bowel Dis, 16(4): 559-567.

DOI: 10.69085/jbb20143261