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Abstract

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T-BET, FOXP3 AND CD8 REGULATING IMMUNE RESPONSE IN COLORECTAL CANCER

Colorectal cancer (CRC) is the third most common cancer diagnosed, and is associated with high rates of propagation and mortality for both men and women. Aimed to to analyze impact of presence of T-bet, Foxp3 and CD8 on colorectal cancer. During the period 1/October/2013 to 1/March/2014, fifty patients with colon cancer (14 female and 36 male) (7-75) years were taken from (Al-Hussain Hospital City/Kerbala, Digestive and Liver Disease /Education Hospital Medical City Baghdad and Teaching Oncology Hospital /Baghdad Medical City/ Baghdad /Iraq).Immunochemical studying of T-bet, Foxp3 and CD8 of colorectal biopsies by using DAKO Company/Denmark. There was a significant increase ($p \leq 0.001$) in the concentration of T-bet, Foxp3 and CD8 respectively in patients male compared with female, also there was a significant variance ($p \leq 0.001$) in the three age groups from (7-20), (21-40) and (41-75) in the levels of T-bet and CD8, whereas a significant difference ($p \leq 0.05$) in the concentration of Foxp3 in the three age groups. This study proved functional role of T-bet, Foxp3 and CD8 in cell-mediated immunity.

Key words: T-bet, Foxp3, CD8, Colorectal cancer.

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Introduction

Colorectal cancer (CRC) is the third most common cancer diagnosed, and is associated with high rates of propagation and mortality for both men and women [1].

Subsets of immune cells can inhibit immunological interaction with tumor cells. The clinical impact of T cells that are supposed to be responsible for the down regulation of a T cell response: regulatory T cells [2].

The mechanisms that inhance a protective T-cell response in the tumor microenvironment remain unclear [3].

Forkhead Box P3 FOXP3, which encodes for helix transcription factor called Scurfin, has been identified to be a key regulatory gene required for the development and functional activity of regulatory T cells. CD4 and CD8, T-cell can suppressed by Tregs that responses to auto- and alloantigens in a contact dependent fashion [4-5].

Studies suggest that T-bet is essential for the development of terminally differentiated CD27, CD11 NK cells [6-7].

A novel transcription factor T-box expressed in T cells (T-bet) behaves as the regulator of Th1 development. Macrophage and antigenic stimulation induce T-bet derived IL-12/IL-18 are important for establishing Th1 mediated immunopathology in CD [8].

Role of CD8+ T cells expressing cytotoxic molecules really spend in a specific immune response against the tumor cells in vivo unclear [9].

Tumor infiltration of CD4+ and CD8+ (T-bet+) effector T cells contribute in the enhancement of T cell activation through T cell receptor stimulation and co-stimulatory signals provides promising strategies for immunotherapy of colon cancer [10].

Aim of study to evaluate the role of T-bet, Foxp3 and CD8 in Patients with Colorectal Cancer.

Patients and Methods

Selection of patients

During the period 1/October/2013 to 1/March/2014, fifty patients with colon cancer (14

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female and 36 male) (7-75) years were taken from (Al-Hussain Hospital City/Kerbala, Digestive and Liver Disease /Education Hospital Medical City Baghdad and Teaching Oncology Hospital /Baghdad Medical City/ Baghdad /Iraq).

Sample collection and assay procedure

Tumors were staged according to the TNM criteria [11]. Known tumor characteristics included differentiation grade.

(Table 1)

The clinical characteristic of patients with colorectal cancer

Characteristic	N	%
No 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
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No of patients	50	100
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Duke A	5	10
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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. De-paraffinzed sections in two changes of xylene of 4 min. each. Xylene 1---xylene 2, re-hydrated sections through graded ethanol of 4 min. each wash in tap water. Antigen unmasked in tris-EDTA buffer pH9 (800 watt) or sodium citrate pH 6(600 watt) and microwaved for 25 min. After cooling for 15 min. for 30 min. Biopsies were washed in deionized water. One – two drops Peroxidase Block were added for 5 min. then washed in PBS for 2x5 min. at room temperature, then washed with PBS1-PBS2. One – two drops Protein Block were added for 5 min. then washed in PBS for 2x5 min. wash PBS1-PBS2. 50 µl was added for each section prepare monoclonal Abs 1 µ l/100 ml PBS (anti-IL-17, anti-perforin and anti-CD68), then incubated for 1 hrs.in room temp, then washed with PBS1-PBS. One drop Post primary Block solution was added for 30 min, then washed with PBS1-PBS2. One drop of Polymer AB secondary Ab was added for 30 min. wash PBS1-PBS2. DAB Buffer 50 µl was added for each section. One µl DAB /50 µl DAB Buffer. Then incubated for 8 min. wash PBS1-PBS2. One drop of Hematoxyline was added for 1 min to 1.5 min, then washed with PBS1-PBS2 and with tap water, one drop of oil-cover was added.

Microscopic analysis

Were calculated with '0' equaling no positive cells,'1'minimal (5%),'2' moderate (5-10%),'3-4' abundant (>10%) quantities of positive cells [12].

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-value ≤ 0.05 was considered significant.

Results

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

There was a significant increase ($p \leq 0.001$) in the concentration of T-bet, Foxp3 and CD8 respectively in patients male compared with female, also there was a significant variance ($p \leq 0.001$) in the three age groups from (7-20), (21-40) and (41-75) in the levels of T-bet and CD8, whereas a significant difference ($p \leq 0.05$) in the concentration of Foxp3 in the three age groups. According to the Duke score, the results revealed a significant difference ($p \leq 0.001$) in the levels of the T-bet, Foxp3 and CD8 in the three stage groups of patients (Table 2, Figure 1).

In (Table 3) there was a highly positive correlation among T-bet with CD8 and Foxp3 ($r=0.7$, $p \leq 0.001$) and ($r= 0.6$, $p \leq 0.001$) respectively. Also, there was a positive correlation ($r=0.6$, $p \leq 0.001$).

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(Table 2)

The levels of T-bet, Foxp3 and CD8 in patients of colorectal cancer.

Characteristic (No)	T-bet Mean ±SD	p-value	Foxp3 Mean ±SD	p-value	CD8 Mean ±SD	p-value
Gender Male(36) Female(14)	2.03±0.9 0.5±0.4	0.0001	3.05±0.6 1.5±0.5	0.0001	2.8±0.6 1.9±0.6	0.0001
Age (7-20) 3 (21-40)5 (41-75)42	0.33±0.05 0.6±0.5 1.8±1	0.0001	1.6±0.5 2±1 2.7±0.8	0.035	1.7±0.5 2.2±0.8 2.7±0.7	0.0001
TNM Duke A (5) Duke B (28) Duke C (17)	0.2±0.04 1.4±1 2.03±0.8	0.0001	1.4±0.5 2.05±0.8 3.2±0.4	0.0001	1.6±0.5 2.1±0.6 3.03±0.5	0.0001

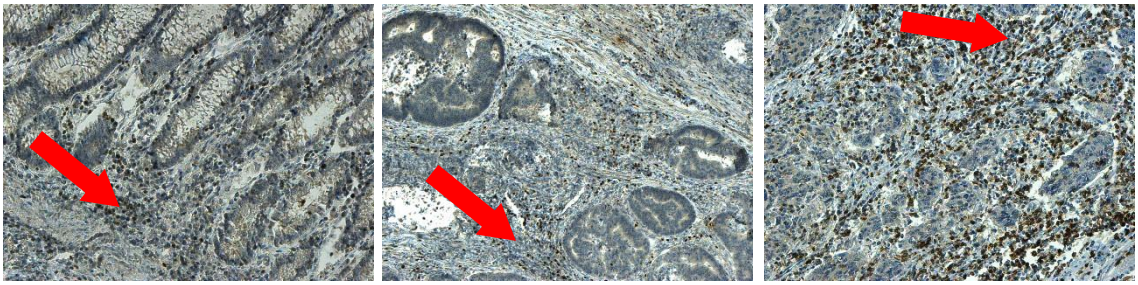
(Table 3)

The correlation of the parameters under study in patients of colorectal cancer

parameters	r	p-value
Tbet vs. Foxp3	0.7	0.0001
Tbet vs. CD8	0.6	0.0001
Foxp3 vs. CD8	0.6	0.0001

Figure 1.

Immunohistochemical staining of colorectal biopsies.



1 2 3

1. Duck/B/T3N0MxStage/2A,
2. Malignant Stage/C/T3N1Mx,
3. Malignant Duck/C1/T3N1Mx/Stage/3A. (Original magnification x400)

Discussion

The current study provided evidence strongly role of Foxp3 expression, T-bet and CD8 in colorectal cancer.

T-bet regulates the relationship between mucosal innate immune cells and the intestinal microbiota that played by T-bet in controlling the profile of cytokines produced by T-cells [13].

T-bet has been suggested to be master regulators of Th1 cells and CD8+ T cells antitumor, their importance for T cell-mediated antitumor immunity is controversial [14].

The presence of FOXP3+Treg cells in colorectal cancer and the relationship of FOXP3 expression with clinicopathological features of colorectal cancer [15].

Presence of Foxp3 cells in tumor tissues shows the interplay between members of the immune system in CRC patients that hypothesized local interactions in the cancer microenvironment between tumor cells and immune cells [2].

FOXP3 appears to have a specific role in the progression of the primary tumour due to its association noted with tumour size, Suggested as biomarkers for prognosis and future immunotherapeutic strategies [16].

FOXP3+Treg cells detection may be useful in prognosis of patients with colorectal cancer [15].

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Researchers suggested CD3, CD4 and CD8 may play a role in the immunopathogenesis of colorectal cancer [17].

Conclusion

Potential functional role of T-bet,Foxp3 and CD8 in colorectal cancer was explained interaction between tumor cells and immune cells that appear immunopathagenesis explanation .This study recommend more immunogenetic studies dehisce new prospects to testing a better therapeutics for colorectal cancer.

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