New York Science Journal

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The Prognostic Significance of TRAP1 and KAI1/CD82 Immunohistochemical Expression in Colorectal Carcinoma

Reham H. Elkady, M.Sc.; Noha M. El-Anwar, M.D.; Hanan A. Al Shenawy, M.D. and Faika A. El-Tatawy, M.D.

Department of Pathology, Faculty of Medicine, Tanta University, Egypt.

Abstract: **Background:** Colorectal carcinoma (CRC) is the third among the most commonly diagnosed cancers worldwide. In Egypt, CRC is the 7th most common cancer. Several proteins are associated with the development and progression of colorectal carcinoma including TRAP1 and KAI1/CD82 proteins. However, it is still controversial whether TRAP1 and KAI1/CD82 expression can be regarded as prognostic factors for colorectal carcinoma patients. **Aim of the Work:** The aim of this work is to study the immunohistochemical expression of TRAP1 and KAI1/CD82 in CRC and evaluate the relationship between their expression in CRC with the available prognostic parameters such as grading, staging, vascular and perineural invasion in the studied cases in order to evaluate their significance in prognosis. **Materials and Methods:** The immunohistochemical expression of TRAP1 and KAI1/CD82 markers was evaluated in 73 cases of colorectal carcinoma. **Results:** TRAP1 expression in colorectal carcinoma showed statistically significant positive relation with tumor histopathological grade, depth of tumor invasion, lymph node status, tumor stage, vascular and perineural invasion. KAI1/CD82 expression in colorectal carcinoma showed statistically significant inverse relation with the histopathological grade, depth of tumor invasion, lymph node status, distant metastasis, tumor stage, vascular and perineural invasion. There was a statistically significant negative correlation between the immunohistochemical expression of TRAP1 and KAI1/CD82 in colorectal carcinoma. **Conclusions and Recommendations:** Combined high expression of TRAP1 with loss of KAI1/CD82 expression suggests poor prognosis and high risk of metastasis in CRC patients. Further studies are needed to confirm the significance of combined use of TRAP1 and KAI1/CD82 expressions in the modulation of treatment regimens in CRC.
[Reham H. Elkady; Noha M. El-Anwar; Hanan A. Al Shenawy and Faika A. El-Tatawy. **The Prognostic Significance of TRAP1 and KAI1/CD82 Immunohistochemical Expression in Colorectal Carcinoma.** *N Y Sci J* 2020;13(2):18-32]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 2. doi:10.7537/marsnys130220.02.

Key Words: TRAP1, KAI1/CD82, Colorectal carcinoma, Prognosis.

1. Introduction

Colorectal carcinoma (CRC) is the third among the most commonly diagnosed cancers worldwide [1]. It represents ~ 10% of the global incidence of cancer [2], and the fourth leading cause of cancer mortality [3]. It is the third most common cancer in males (10.0% of the total) and the second in females (9.2% of the total) worldwide [4].

CRC is the 7th most common cancer in Egypt, representing 3.47% of male cancers and 3% of female cancers [5]. The latest statistics by Gharbiah cancer registry was in 2007, published in Volume X of Cancer Incidence in Five Continents, showed that CRC cases represent nearly 4.7% of all cancer cases in males and 4.4% in females [6,7].

CRC is a consequence of an array of factors, which may be inherited or acquired through life course. Economic development and westernization of lifestyle had increased the exposure to certain environmental and lifestyle factors, increasing the risk of developing the disease [3]. At the same time, CRC is by far the most curable gastrointestinal carcinoma. The mean age at diagnosis is in the sixth to seventh decades of life [8]. However, increasing rate of CRC in young adults is observed recently, due to unhealthy dietary choices and red meat consumption, physical inactivity and obesity, besides, many inherited syndromes [9].

Adenocarcinomas make up 95 % of all colorectal cancer cases. Other rare types include neuroendocrine, squamous cell, adenosquamous, spindle cell and undifferentiated colorectal carcinomas [10]. CRC is a genetically heterogeneous and complicated disease. Numerous therapeutic regimens together with target therapies for CRCs have been proposed, but with all the currently approved standard therapies, the disease is still progressive for most of patients [11]. Tumor metastasis is the major factor that worsens the prognosis of CRC. The progression of CRC is particularly associated with the mutation of various

molecules, but few are used to predict metastasis in CRC [12].

Spread of tumor cells from a primary tumor to secondary sites within the body is complex, involving processes such as epithelial-mesenchymal transition, cell migration, invasion, adhesion, proliferation, and angiogenesis [13]. Therefore, a primary challenge is to develop improved methods to predict the metastatic potential of tumor cells.

Heat shock proteins (HSPs) are a family of proteins, found in virtually all living organisms, produced by cells in response to stressful conditions such as heat shock [14]. Many of them are molecular chaperones that play a role in development, stress responses and diseases including cancer [15]. They are named according to their molecular weight, for example; Hsp60, Hsp70, and Hsp90 (the most widely studied HSPs) refer to families of heat shock proteins of 60, 70, and 90 kilodaltons in size, respectively [16].

Tumor necrosis factor receptor-associated protein 1 (TRAP1) is a molecular chaperone of (HSP90) family, first identified in 1995 by Song et al. as a HSP90 family member, associated with the type 1 tumor necrosis factor receptor-1 (TNFR1) [17]. It is encoded by a gene, located on chromosome 16 and the protein is mostly localized to the mitochondrial matrix. Biologically, TRAP1 modulates the permeability transition pore of the mitochondrial inner membrane and protects the mitochondrial structure from excessive reactive oxygen species (ROS) induced cell death, such as occurring in cancers [18]. Also, TRAP1 plays many roles in cancer cells, i.e. protection from stress and apoptosis [19], maintenance of stemness [20], protein homeostasis [21], intracellular signaling, cell migration [22], cell cycle dysregulation [23], induction of drug resistance [24] and bioenergetics [25].

Recent studies showed that TRAP1 plays a regulatory function on the BRAF pathway synthesis/ubiquitination, which may explain its role in drug-resistant cases. As human BRAF-driven tumors are aggressive malignancies with poor clinical outcome and lack of sensitivity to therapies [26]. Other studies showed that TRAP1 may be associated with positive lymph node metastasis [27] and a shorter median overall survival in CRC [28]. But there are still limited studies about the relationship between TRAP1 expression and pathologic parameters in CRCs.

Tetraspanins are a family of integral membrane proteins with four membrane-spanning domains. They form large multimeric complexes that consist of tetraspanins as well as other membrane and cytosolic proteins such as receptor tyrosine kinases, integrins, and adaptor proteins that are integral to signaling cascades [29]. Recent studies have discovered the importance of tetraspanins in solid tumors and hematological malignancies. It has the ability to control the regulation of cell migration, adhesion, differentiation, and proliferation [30]. Some members of this family are known as metastasis suppressor genes, while others are supposed to promote tumor progression [31].

Tetraspanin CD82, also known as Kangai 1 (KAI1), located on chromosome 11p11.2., first recognized in 1995 by Dong et al., as a suppressor gene of metastasis in prostate cancer cells, then found that KAI1/CD82 also suppresses the invasion and/or metastasis of other epithelial malignancies [31,32]. Its differential expressions were found in various normal and malignant tissues, which indicate that KAI1/CD82 may play a pivotal role in cancer growth, progression, motility, invasion, and metastasis [33].

KAI1/CD82 appears to inhibit multiple steps of the metastatic cascade including cell motility and invasion, proliferation, apoptosis and induce senescence. It has an impact on the cell-cell or cell-extracellular matrix interactions in ways that are non-permissive for survival and proliferation beyond the primary tumor. This broad range of effects can be achieved by the modulation of the activity and trafficking of proteins critical for metastasis through physical or functional interactions with KAI1/CD82 [29,34]. So, KAI1/CD82 was identified as a useful biomarker for metastasis and prognosis in diverse human cancers [35].

2. Materials and Methods

This study was a retrospective and prospective study, carried out on 73 cases primarily diagnosed as colorectal carcinoma in colectomy specimens collected during the period of research from the Pathology Department, Faculty of Medicine, Tanta University. Patients' data were obtained from the cases' clinical sheets. The gross picture, location, size of the tumors and presence of distant metastasis of the available cases were obtained from the pathology files (for retrospective cases) and by gross examination of fresh speccimens (for prospective cases). Approval from the research ethics committee (REC), Faculty of Medicine, Tanta University was taken before conducting the study.

Cases were classified microscopically according to the fourth edition of the World Health Organization (WHO) classification system, 2010 [36]. Colorectal adenocarcinoma cases were graded according to WHO, based on the degree of gland formation into well differentiated (GI), moderately differentiated (GII), poorly differentiated (GIII) and undifferentiated (GIV) [37].

		-The majority (>75%) of glands are smooth and regular. -No significant component of high- grade nuclei
GII	Moderately differentiated	50-95% gland formation
GIII	Poorly differentiated	<50% gland formation
GIV	Undifferentiated	No apparent gland formation

Mucinous carcinomas and signet ring cell carcinomas are considered to be grade III because they have much worse prognosis from classic adenocarcinomas [38]. Both large and small cell neuroendocrine carcinomas belong to high-grade tumors as recent studies revealed that more than 50% of their cases were found to have liver, bone or nodal metastasis at the time of diagnosis even when the tumor was microscopic [39]. The biological behavior of adenosquamous carcinoma is determined by the degree of differentiation of the glandular component [8,40]. Pathological staging of the studied CRC cases was determined according to the recommendations of the 8th edition of AJCC, Cancer Staging Manual, 2017 by using the TNM staging system [41].

Immunohistochemical staining was performed on 10% formalin fixed, paraffin embedded tissue blocks for evaluation of TRAP1 and KAI1/CD82 expression. Sections were labeled, using primary antibodies to TRAP1 (Rabbit polyclonal antibody, Kit no. GTX102017, GeneTex, USA, dilution 1:300) and KAI1/CD82 (Rabbit polyclonal antibody, Kit no. GTX100633, GeneTex, USA, dilution 1:150). TRAP1 positive staining was defined as cytoplasmic staining of epithelial cells. TRAP1 expression was evaluated, according to reported procedures [11], for each tissue sample by calculating a total immunohistochemistry score as the product of a proportion and intensity score. The proportion score described the estimated fraction of positively stained tumor cells (0 = none; 1 = 1 ~ 25%; 2 = 26 ~ 50%; 3 = 51 ~ 75%; 4 = 76 ~ 100%). The intensity score represented the estimated staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) of the samples. The final immunohistochemistry score ranged from 0 to 12. The high-TRAP1 expression group was arbitrarily defined as a total score ≥ 6, while the low-TRAP1 expression group was considered as a total score < 6. TRAP1 is undetectable or expressed at very low levels in normal tissue [42]. KAI1/CD82 positive staining was mainly confined in the membrane and/or cytoplasm of the cancer cell. According to reported procedures [12,43], the immunostaining, for each tissue sample, was graded in terms of both extent and intensity. The intensity of the staining was divided into four grades: 0, none; 1, weak; 2, moderate; 3, strong. The extent of staining was also divided into five categories: 0, ≤5 %; 1, 6–25 %; 2, 26–50 %; 3, 51–75 %; 4, 76–100 %. Finally, we determined the score by multiplying the intensity and the extent of staining to produce a range of immunostaining scores from 0 to 12. The immunostaining was considered positive when the scores were ≥3. Normal colonic mucosa showed positive cytoplasmic staining for KAI1/CD82, so, sections of normal colonic mucosa were used as a positive internal control for the immunoreaction [43].

The collected data were organized, tabulated and statistically analyzed using SPSS, version 23. Chi-square test and Monte Carlo test were used to assess immunohistochemical markers expression with respect to available clinicopathologic parameters. Significance was adopted at p-value <0.05 for interpretation of results of tests of significance. The correlations among TRAP1 and KAI1/CD82 were compared using Spearman's coefficient test.

3. Results

The clinicopathological characteristics of the studied cases were summarized in (Table 1). We evaluated 73 cases of colorectal carcinoma for immunohistochemical TRAP1 and KAI1/CD82 expression and evaluated the relation with different clinicopathological characteristics (Tables 2,3).

Among 73 studied colorectal carcinoma cases, 52 cases (71.2%) showed high TRAP1 expression. The TRAP1 immunohistochemical score in the studied CRC cases showed statistically significant positive relation with tumor grade (*p*- value= 0.001), depth of invasion (*p*-value= 0.043), lymph node status (*p*-value= 0.014), TNM stage (*p*- value= 0.008), vascular (*p*-value= 0.033) and perineural invasion (*p*-value= 0.041), but didn't show statistically significant relation with histopathological type and presence of distant metastasis (*p*-value= 0.913 and 0.213 respectively).

Among 73 studied colorectal carcinoma cases, 29 cases (39.7%) showed positive KAI1/CD82 expression while 44 cases (60.3%) showed negative KAI1/CD82 expression. The KAI1/CD82 immunohistochemical expression in the studied CRC cases showed statistically significant inverse relation with tumor grade (*p*- value= 0.002), depth of invasion (*p*-value= 0.009), lymph node status (*p*-value= 0.019), presence of distant metastasis (*p*-value= 0.034), TNM stage (*p*-value= 0.002), vascular (*p*-value= 0.002) and perineural invasion (*p*-value= 0.014), but didn't show statistically significant relation with histopathological type (*p*-value= 0.129). As regards to this study, there was a significant negative correlation between TRAP1 immunohistochemical expression score and KAI1/CD82 immunohistochemical expression in

studied colorectal carcinoma cases (r = - 0.515, *p*- value <0.001).

Table (1): The clinicopathological features of the studied cases

Clinicopathological features	No.	%
1- Age		
• ≤60	46	63
• >60	27	37
2- Sex		
• Male	41	56.2
• Female	32	43.8
3- Size		
• ≤5cm	32	43.8
• >5cm	41	56.2
4- Site		
• Right Colon	30	41.1
• Left Colon	21	28.8
• Rectum	22	30.1
5- Gross configuration		
• Fungating mass	45	61.6
• Ulcerating	14	19.2
• Diffusely infiltrating	14	19.2
6- Histopathological types		
➢ Adenocarcinomas;		
• Conventional adenocarcinoma	33	45.2
• Conventional Adenocarcinoma with focal mucoid changes	9	12.3
• Mucinous carcinoma	13	17.8
• Signet ring cell carcinoma	6	8.2
➢ Carcinomas with neuroendocrine differentiation;		
• Adenocarcinoma with neuroendocrine differentiation	7	9.5
• Small cell neuroendocrine carcinoma	1	1.4
• Large cell neuroendocrine carcinoma	1	1.4
➢ Adenosquamous carcinoma	2	2.7
➢ Adenocarcinoma with trophoblastic differentiation	1	1.4
7- Histopathological grading		
• Grade I	10	13.7
• Grade II	30	41.1
• Grade III	33	45.2
8- Lymphovascular invasion		
• Presence.	29	39.7
• Absence.	44	60.3
9- Perineural invasion		
• Presence	20	27.4
• Absence	5	72.6
10- Depth of invasion (T stage)		
• T1	2	2.7
• T2	5	6.8
• T3	40	54.8
• T4a	22	30.1
• T4b	4	5.5
11- Lymph node status (N stage)		
• N0	23	31.5
• N1a	10	13.7

• N1b	14	19.2
• N1c	2	2.7
• N2a	12	16.4
• N2b	12	16.4
12-Distant metastasis (M stage)		
• Mx	60	82.2
• M1a	4	5.5
• M1c	9	12.3
13- TNM stage grouping		
• Stage I.	5	6.8
• Stage IIA.	11	15.1
• Stage IIB.	5	6.8
• Stage IIIA.	2	2.7
• Stage IIIB.	28	38.4
• Stage IIIC.	9	12.3
• Stage IVA.	4	5.5
• Stage IVC.	9	12.3

Table (2): Relation between TRAP1 immunohistochemical score and different clinicopathological parameters

variables		TRAP1 expression		Total	X^2	p
		High	Low			
Histopathological types						
➢ *Adenocarcinoma;*	N	43	18	61	3.282	0.913
	%	70.5	29.5	100		
• Conventional adenocarcinoma	N	21	12	33		
	%	63.6%	36.4%	100%		
• Conventional adenocarcinoma with mucoid changes.	N	7	2	9		
	%	77.8	22.2	100%		
• Mucinous carcinoma	N	10	3	13		
	%	76.9%	23.1%	100%		
• Signet ring carcinoma	N	5	1	6		
	%	83.3%	16.7%	100%		
➢ *Carcinomas with neuroendocrine differentiation;*	N	7	2	9		
	%	77.8	22.2	100		
• Adenocarcinoma with neuroendocrine diff.	N	5	2	7		
	%	71.4%	28.6%	100%		
• Small cell neuroendocrine carcinoma	N	1	0	1		
	%					
• Large cell neuroendocrine carcinoma	N	1	0	1		
	%					
➢ *Adenosquamous carcinoma*	N	1	1	2		
	%					
➢ *Adenocarcinoma with trophoblastic differentiation*	N	1	0	1		
	%					
Histopathological grades						
• GI	N	2	8	10	15.046	0.001
	%	20	80	100		
• GII	N	23	7	30		
	%	76.7	23.3	100		
• GIII	N	27	6	33		
	%	81.8	18.2	100		
Depth of invasion (T) status						
• T1	N	0	2	2	8.579	0.043
	%	0	100	100		
• T2	N	2	3	5		
	%	40	60	100		
	N	28	12	40		

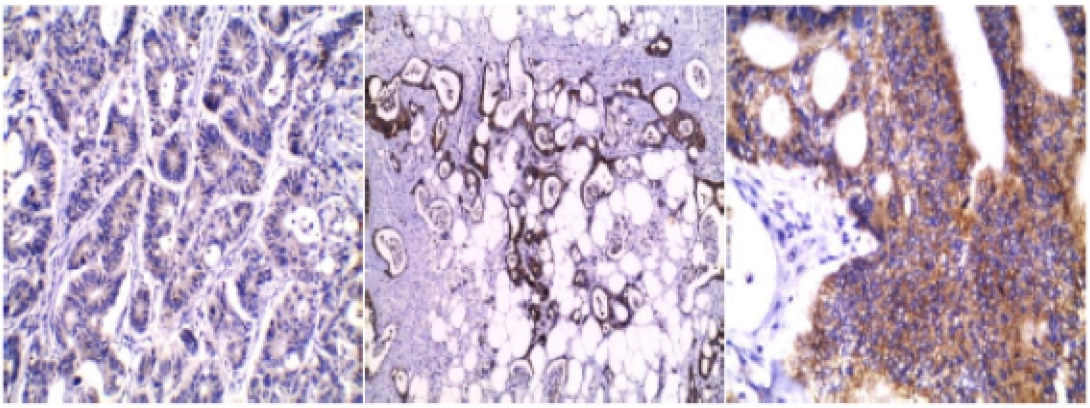
• T3	%	70	30	100		
• T4a	N	18	4	22		
	%	81.8	18.2	100		
• T4b	N	4	0	4		
	%	100	0	100		
Lymph node status (N) status						
➢ N0	N	10	13	23	13.133	0.014
	%	43.5	56.5	100		
➢ N1	N	20	6	26		
	%	77	23	100		
• N1a	N	7	3	10		
	%	70	30	100		
• N1b	N	11	3	14		
	%	78.6	21.4	100		
• N1c	N	2	0	2		
	%	100	0	100		
➢ N2	N	22	2	24		
	%	91.7	8.3	100		
• N2a	N	11	1	12		
	%	91.7	8.3	100		
• N2b	N	11	1	12		
	%	91.7	8.3	100		
Distant metastasis (M) status						
➢ Mx	N	40	20	60	2.825	0.213
	%	66.7	33.3	100		
➢ M1	N	12	1	13		
	%	92.3	7.7	100		
• M1a	N	4	0	4		
	%	100	0	100		
• M1c	N	8	1	9		
	%	88.9	11.1	100		
TNM staging groups						
➢ Stage I	N	0	5	5	16.520	0.008
	%	0	100	100		
➢ Stage II	N	9	7	16		
	%	56.2	43.8	100		
• IIA	N	6	5	11		
	%	54.5	45.5	100		
• IIB	N	3	2	5		
	%	60	40	100		
➢ Stage III	N	31	8	39		
	%	79.5	20.5	100		
• IIIA	N	2	0	2		
	%	100	0	100		
• IIIB	N	21	7	28		
	%	75	25	100		
• IIIC	N	8	1	9		
	%	88.9	11.1	100		
➢ Stage IV	N	12	1	13		
	%	92.3	7.7	100		
• IVA	N	4	0	4		
	%	100	0	100		
• IVB	N	8	1	9		
	%	88.9	11.1	100		
Vascular invasion						
Present	N	25	4	29	5.265	0.033
	%	86.2	13.8	100		
Absent	N	27	17	44		

	%	61.4	38.6	100		
Perineural invasion						
Present	N	18	2	20	4.735	0.041
	%	90	10	100		
Absent	N	34	19	53		
	%	64.2	35.8	100		
Total	N	52	21	73		
	%	71.2%	28.8%	100%		

Table (3): Relation between KAI1/CD82 immunohistochemical expression and different clinicopathological parameters

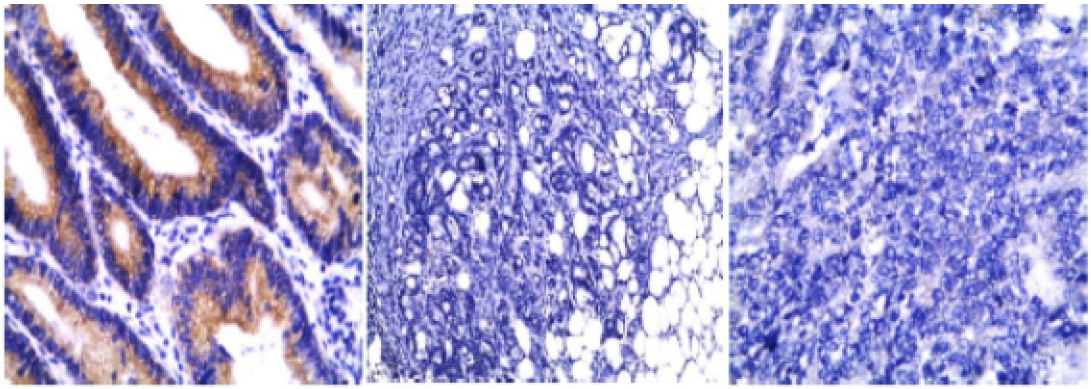
variables		KAI1/CD82 expression		Total	X^2	p
		positive	negative			
Histopathological types						
➢ *Adenocarcinomas;*	N	26	35	61	10.072	0.129
	%	42.6%	57.4%	100%		
• Conventional adenocarcinoma	N	15	18	33		
	%	45.5%	54.5%	100%		
• Adenocarcinoma with mucoid changes.	N	6	3	9		
	%	66.7%	33.3%	100%		
• Mucinous carcinoma	N	5	8	13		
	%	38.5%	61.5%	100%		
• Signet ring carcinoma	N	0	6	6		
	%	0%	100%	100%		
➢ *Carcinomas with neuroendocrine differentiation*	N	2	7	9		
	%	22.2%	77.8%	100%		
• Conventional adenocarcinoma with neuroendocrine diff.	N	1	6	7		
	%	14.3%	85.7%	100%		
• Small cell neuroendocrine carcinoma	N	0	1	1		
	%					
• Large cell neuroendocrine carcinoma	N	1	0	1		
	%					
➢ *Adenosquamous carcinoma*	N	1	1	2		
	%					
➢ *Adenocarcinoma with trophoblastic diff.*	N	0	1	1		
	%					
Histopathological grades						
• GI	N	8	2	10	12.101	0.002
	%	80%	20%	100%		
• GII	N	14	16	30		
	%	46.7	53.3	100		
• GIII	N	7	26	33		
	%	21.2	78.8	100		
Depth of invasion (T) status						
• T1	N	2	0	2	11.093	0.009
	%	100	0	100		
• T2	N	4	1	5		
	%	80	20	100		
• T3	N	18	22	40		
	%	45	55	100		
• T4a	N	5	17	22		
	%	22.7	77.3	100		
• T4b	N	0	4	4		
	%	0	100	100		
Lymph node status (N) status						
➢ N0	N	15	8	23	12.415	0.019
	%	65.2	34.8	100		

➢ N1	N	9	17	26		
	%	34.6	65.4	100		
• N1a	N	4	6	10		
	%	40	60	100		
• N1b	N	4	10	14		
	%	28.6	71.4	100		
• N1c	N	1	1	2		
	%	50	50	100		
➢ N2	N	5	19	24		
	%	20.8	79.2	100		
• N2a	N	4	8	12		
	%	33.3	66.7	100		
• N2b	N	1	11	12		
	%	8.3	91.7	100		
Distant metastasis (M) status						
➢ Mx	N	28	32	60	6.429	0.034
	%	46.7	53.3	100		
➢ M1	N	1	12	13		
	%	7.7	92.3	100		
• M1a	N	0	4	4		
	%	0	100	100		
• M1c	N	1	8	9		
	%	11.1	88.9	100		
TNM staging groups						
➢ Stage I	N	5	0	5	20.641	0.002
	%	100	0	100		
➢ Stage II	N	10	6	16		
	%	62.5	37.5	100		
• IIA	N	8	3	11		
	%	72.7	27.3	100		
• IIB	N	2	3	5		
	%	40	60	100		
➢ Stage III	N	13	26	39		
	%	33.3	66.7	100		
• IIIA	N	1	1	2		
	%	50	50	100		
• IIIB	N	11	17	28		
	%	39.3	60.7	100		
• IIIC	N	1	8	9		
	%	11.1	88.9	100		
➢ Stage IV	N	1	12	13		
	%	7.7	92.3	100		
• IVA	N	0	4	4		
	%	0	100	100		
• IVB	N	1	8	9		
	%	11.1	88.9	100		
Vascular invasion						
Present	N	5	24	29	10.159	.002
	%	17.2	82.8	100		
Absent	N	24	20	44		
	%	54.5	45.5	100		
Perineural invasion						
Present	N	3	17	20	7.034	0.014
	%	15	85	100		
Absent	N	26	27	53		
	%	49.1	50.9	100		
Total	N	29	44	73		
	%	39.7%	60.3%	100.0%		



(A) (B) (C)

Fig. (1): (1A) Conventional adenocarcinoma (grade I) showing low cytoplasmic TRAP1 expression (X200). (1B) Conventional adenocarcinoma (grade II), invading serosal fat, showing high cytoplasmic TRAP1 expression (X100). (1C) Conventional adenocarcinoma (grade III) showing high cytoplasmic TRAP1 expression (X400).



(A) (B) (C)

Fig. (2): (A) Conventional adenocarcinoma (grade I) showing positive cytoplasmic KAI1/CD82 expression (X400). (B) Conventional adenocarcinoma (grade II), invading serosal fat, showing negative cytoplasmic KAI1/CD82 expression (X100). (C) Conventional adenocarcinoma (grade III) showing negative cytoplasmic KAI1/CD82 expression (X400).

4. Discussion

TRAP1 is a molecular chaperone of (HSP90) family, associated with the type 1 tumor necrosis factor receptor-1 (TNFR1) [17]. It is encoded by a gene, located on chromosome 16 and the protein is mostly localized to the mitochondrial matrix. It modulates the permeability transition pore of the mitochondrial inner membrane and protects the mitochondria from excessive reactive oxygen species (ROS) induced cell death [18]. TRAP1 is upregulated in several human malignancies [44,45], including CRC [24]. High TRAP1 levels have been proposed as a prognostic biomarker in this malignancy, being associated with extensive lymph node dissemination [27] and with poor overall survival (OS) in metastatic disease [28].

In our study, high TRAP1 immunohistochemical expression score was noted in the studied CRC cases with advanced tumor grade, increased depth of invasion, positive lymph node metastasis, advanced TNM stage and presence of vascular and perineural invasion. From that, we conclude that TRAP1 expression may be a poor prognostic marker in colorectal carcinoma. Similarly, Gao et al., [27]; Maddalena et al., [46]; Pak et al., [11]; Bărbălan et al., [47] and Gao et al., [48] found that TRAP1 expression is a useful poor prognostic marker in CRC.

Costantino et al., [24] reported that TRAP1 expression was contributed to multi-drug resistance and suppressing apoptosis in human CRC cells. Han et al., [28] reported that CRC patients with positive.

TRAP1 expression had poorer prognosis. In addition, multiple studies have been successful in suggesting the role of TRAP1 as a clinical biomarker and therapeutic target in cancer especially BRAF

mutated advanced CRC, improving response to chemotherapy resulting in better survival rates [49,50].

According to our study, there was a statistically significant relation between TRAP1 immunohistochemical expression and the histopathological grade of the tumor. There was high TRAP1 expression in 81.8% of cases of grade III and only 20% of cases of grade I. Similar results were detected by Pak et al., [11] and Gao et al., [48]. The upregulation of TRAP1 in high-grade tumors clarified its major role in crucial biological functions that influence various aspects of cell physiology, including proliferation, apoptosis, differentiation, and morphogenesis. It is also significantly involved in cell adhesion and motility, cancer invasion and metastasis [51,52]. In contrast, Gao et al., [27] and Maddalena et al., [46] who reported no significant relation between TRAP1 expression and tumor differentiation among their studied CRC cases.

TRAP1 expression was observed to increase with increased depth of the tumor invasion (T status) and the relation between TRAP1 expression score and depth of invasion (T) in the studied CRC cases was statistically significant. Similar results were reported by Gao et al., [27]; Pak et al., [11]; Gao et al., [48] who reported that TRAP1 expression was significantly associated with infiltration depth in their studied CRC cases. In contrast, Maddalena et al., [46] found no significant relation between TRAP1 expression and depth of invasion in CRC cases. Several studies have identified that TRAP1 is abundantly localized in the mitochondria of tumor cells [44,53] and is involved in protecting against oxidative stress and apoptosis. Furthermore, it has been reported that TRAP1 function synergistically with tumor necrosis factor receptor 1 to modulate the expression of the cell adhesion molecule N cadherin, altering the intercellular adhesion of cells [54]. This demonstrates the role of TRAP1 in the processes of cell invasion and motility, which are characteristics of tumorigenesis and metastatic spread.

Pak et al., [11] suggest that TRAP1 enables tumor cells to invade stromal tissue by epithelial-mesenchymal transition (EMT). Also, TNF-α promotes tumor invasion via induction of matrix metalloproteinases, and finally modulates EMT in a model of CRC [55]. In addition, TRAP1 inhibits the enzymatic activity of succinate dehydrogenase (SDH), and SDH inhibition leads to succinate- dependent hypoxia-inducible factor 1-alpha (HIF1- a) stabilization [56]. HIF1 stabilization contributes to neoplastic processes by EMT [57] and EMT plays a critical role in the migration of tumor cells from the primary site into stromal tissue [58].

Gao et al., [48] also found gradually increased TRAP1 expression levels from the colorectal mucosa of high-grade intraepithelial neoplasia to CRC. This suggests that TRAP1 expression may be detected at the earliest stage of CRC tumorigenesis, and TRAP1 may serve a function not only in the progression, but also in the onset of malignancy, and may be gradually activated during colorectal carcinogenesis. The precise pathologic mechanisms for TRAP1 in promoting cancer invasion are still not fully understood and many questions remain to be answered. But TRAP1 seems to be one of the critical players in biologic processes of tumor invasion in CRC [11].

TRAP1 expression was observed to increase with increased lymph node metastasis (N status) and the relation between TRAP1 expression score and lymph node metastasis (N) in the studied CRC cases was statistically significant. Similar results were reported by Gao et al., [27] and Gao et al., [48] who reported that TRAP1 expression was significantly associated with lymph node metastasis in CRC. In contrast, Maddalena et al., [46] found no significant relation between TRAP1 expression and lymph node metastasis in CRC. Gao et al., [27] suggested that TRAP1 plays an important role in the progression of CRC from a localized to lymph node metastatic disease. In turn, TRAP1 expression may serve as a molecular marker for lymph node metastasis and poor prognosis [48].

TRAP1 immunohistochemical score was observed to increase with increased TNM stage of the tumor and the relation between TRAP1 expression score and tumor TNM stage in the studied CRC cases was statistically significant. Similar results were detected by Gao et al., [27] and Gao et al., [48] who reported a statistically significant relation between TRAP1 expression and tumor TNM stage. In contrast, Maddalena et al., [46] and Pak et al., [11] detected no significant relation between TRAP1 expression and tumor TNM stage.

According to the current study, the relation between TRAP1 score and lymphovascular invasion in the studied CRC cases was statistically significant and the relation observed between TRAP1 expression score and perineural invasion in the studied CRC cases was also statistically significant. These results may be explained by the same mechanisms of TRAP1 mediated tumor invasion and by taking into account that lymphovascular invasion is a step, through which, metastasis occurs. In contrast, Pak et al., [11] reported no significant relation between TRAP1 expression and lymphovascular invasion.

KAI1, also named as CD82, one of the tetraspanin superfamily (TM4SF), most of which have four transmembrane domains [59], located on chromosome 11p11.2., first recognized as a suppressor gene of metastasis in prostate cancer cells [31], then found that KAI1/CD82 expression also suppresses the invasion and/or metastasis of other epithelial

malignancies. Metastasis is inhibited by multiple mechanisms such as inhibition of cell motility and invasion, promotion of apoptosis, induction of the senescence in tumor cells, as well as secretion of the external ß-catenin [29]. In addition, other studies revealed that reduced KAI1/CD82 expression is associated with altered adhesion to specific components of the extracellular matrix such as fibronectin, reduced cell–cell interactions, and increased cell motility, leading to a more invasive and metastatic ability [32].

Current understanding of KAI1/CD82 function indicates that it is likely to be involved in detachment, motility/invasion, and cell survival. KAI1/CD82 can interact with other tetraspanin proteins (e.g., CD151 and CD81), integrins (e.g., α3β1, α4β1, and α5β1), receptor tyrosine kinases (e.g., epithelial growth factor receptor [EGFR] and c-Met), and chemokines to regulate the migration, adhesion, and signaling of cells [60].

According to our study, negative KAI1/CD82 immunohistochemical expression in the studied CRC cases was noted with advanced tumor grade, increased depth of invasion, positive lymph node metastasis, presence of distant metastasis, advanced TNM stage and presence of vascular or perineural invasion. These significant relationships between negative KAI1/CD82 expression and poor prognosis in CRC may indicate that KAI1/CD82 could be a promising biomarker for predicting the infiltration, metastasis, and prognosis of CRC, and the biological functions of KAI1/CD82 are of great research value of the subject. Similarly, Lombardi et al., [61]; Maurer et al., [62]; Hashida et al., [63]; Wu et al., [12]; Zhu et al., [31] and Ganji et al., [64] found that KAI1/CD82 expression is a useful good prognostic marker in CRC. Ma et al., [65] stated the association of KAI1/CD82 gene polymorphisms with CRCs susceptibility. Hashida et al., [63] observed that the survival rate for colon cancer patients with negative KAI1/CD82 expression was strikingly lower than that of patients with KAI1/CD82-positive tumors. In addition, Wu et al., [66] reported that various chemotherapeutic drugs, such as VP 16, can effectively upregulate KAI1/CD82 protein expression, which provided reliable proof for clinical therapy of metastasis.

According to our study, there was a statistically significant relation between KAI1/CD82 immunohistochemical expression and the histopathological grade of the tumor. There was positive KAI1/CD82 expression in 80% of cases of grade I and only 21.2% of cases of grade III. Similar results were detected by Wu et al., [12] and Zhu et al., [43]. In contrast, Hashida et al., [63] reported no significant relation between KAI1/CD82 expression and tumor differentiation among their studied CRC cases.

Reduced KAI1/CD82 expression was observed with increased depth of the tumor invasion (T status) and the relation between KAI1/CD82 expression score and depth of invasion (T) in the studied CRC cases was statistically significant. Similar results were reported by Maurer et al., [62]; Wu et al., [12] and Zhu et al., [43] who reported that KAI1/CD82 expression was significantly reduced with tumor progression in CRC. In contrast, Hashida et al., [63] found no significant relation between KAI1/CD82 expression and depth of invasion in CRC. Previous studies have identified that high KAI1/CD82 expression suppresses the development of a motile mesenchymal phenotype and rather intensifies epithelial characteristics in human prostate cancer cells adhered to the fibronectin matrix. KAI1/CD82 inhibits integrin-mediated intracellular signaling cascades. Thus, KAI1/CD82 represses the matrix-binding affinity and signaling activity of the integrins, resulting in reduced integrin-matrix interactions and integrin outside-in signaling [60].

According to the current study, KAI1/CD82 expression was decreasing up to be completely lost with increased lymph node metastasis (N status) and the relation between KAI1/CD82 expression score and lymph node metastasis (N) in the studied CRC cases was statistically significant. Similar results were reported by Maurer et al., [62]; Hashida et al., [63]; Wu et al., [12]; Zhu et al., [43] and Ganji et al., [64] who reported that KAI1/CD82 negative expression was significantly associated with lymph node metastasis in CRC.

According to the current study, KAI1/CD82 expression was observed to decrease in cases with distant metastasis than those with no documented distant metastasis and the relation between KAI1/CD82 expression and distant metastasis (M) in the studied CRC cases was statistically significant. Similar results were detected by Lombardi et al., [61]; Maurer et al., [62]; Wu et al., [12]; Zhu et al., [43] and Ganji et al., [64] who detected significant relation between KAI1/CD82 expression and distant metastasis in CRC cases. While Yang et al., [67] indicated that KAI1/CD82 expression was regained in CRC associated with metastasis.

KAI1/CD82 immunohistochemical score was observed to decrease with increased TNM stage of the tumor and the relation between KAI1/CD82 expression score and tumor TNM stage in the studied CRC cases was statistically significant. Similar results were detected by Hashida et al., [63]; Wu et al., [12]; Zhu et al., [43] and Ganji et al., [64] who reported a statistically significant relation between KAI1/CD82 expression and tumor TNM stage in CRC.

According to the current study, the relation between KAI1/CD82 expression and lymphovascular invasion in the studied CRC cases was statistically significant, which was similar to the results of metanalysis study done by Zhu et al., [31]. As well as, the relation observed between KAI1/CD82 expression score and perineural invasion in the studied CRC cases was also statistically significant.

As regards to this study, there was a significant negative correlation between TRAP1 and KAI1/CD82 immunohistochemical expression. This result may arise from the effect of both molecular markers on invasive properties of CRCs. Since, both act in early tumorigenesis and differentiation of tumor cells, and affect epithelial-mesenchymal transition, either by enhancing mesenchymal phenotype and tumor cell migration (TRAP1) or intensifying epithelial phenotype with inhibition of tumor cell motility, migration and deep invasion (KAI1/CD82).

Therefore, we conclude that combined high expression of TRAP1 with loss of KAI1/CD82 suggests poor prognosis and high risk of metastasis in CRC patients. Therefore, this combination could be used to predict tumor behavior and evaluating the prognosis and screening for patients with a high risk of metastasis.

Recommendations:

Both TRAP1 and KAI1/CD82 can be promising therapeutic targets to decrease metastatic potential in CRC patients. So, more studies are recommended to investigate the role of TRAP1 inhibitors as chemotherapeutic agents in colorectal carcinoma and further defining the relationship between targeted drug therapy and expression of TRAP1 in colorectal cancer. Further studies should be carried out for identification of KAI1/CD82 down-regulatory mechanisms in order to be able to develop new therapeutic targets via enhancing this inhibitory action against CRC.

Conflict of interest:

None declared.

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