

Expression Levels of the *CA9*, *WT1*, and *PRAME* Genes and Genotyping-Associated Antigens for the Diagnosis and Prognosis of Colorectal Cancer

Tabark S Jassim¹

¹Prosthodontic Technology Department, Dijlah University College, Baghdad, Iraq

Address for correspondence Tabark S Jassim, PhD, Prosthodontic Technology Department, Dijlah University College, Baghdad, Zipcode: 10022, Baghdad, Iraq (e-mail: rawaqjasim@gmail.com).

J Coloproctol 2022;42(2):120–125.

Abstract

Background Colorectal cancer (CRC) is the third most prevalent type of cancer worldwide, and is one of the major health problems in Asia, Africa, Europe, and America. The tumor antigens recently are of interesting indicators as diagnostic and prognostic tools, The aim of the present study is to detect the expression levels of *carbonic anhydrase IX (CA9)*, the *Wilms tumor gene (WT1)*, and the *preferentially expressed antigen in melanoma (PRAME)* in the peripheral blood of CRC patients in comparison with healthy controls.

Methods A prospective case-control study of CRC patients was conducted. We included 25 newly-diagnosed CRC eligible patients and obtained peripheral blood samples of them as well as 10 blood samples from the control group. All samples were then submitted to deoxyribonucleic acid (DNA) extraction and a molecular study through real-time polymerase chain reaction (PCR).

Results The CRC group consisted of 15 (60%) female and 10 (40%) male patients with a mean age of 50.52 ± 9.8 years, while the control group included 4 (40%) female and 6 (60%) male patients with a mean age of 47.7 ± 7.9 years. The CRC group, 24 (96%) of patient samples were *CA9*-positive with strong statistically significant differences ($p < 0.00001$; sensitivity: 96%; specificity: 90%). Regarding the *WT1* gene, there were 11 (44%) positive samples in the CRC group, with no statistically significant differences ($p = 0.055$; sensitivity: 44%; specificity: 90%). The *PRAME* gene was positive in 9 (36%) samples in the CRC group, with no statistically significant differences ($p = 0.357$; sensitivity: 36%; specificity: 80%). Among *CA9* (24 patients; 96%) of patients with CRC expressed positive results, in *WT1* 11(91.6%) CRC patients expressed gene, and in *PRAME* gene, 9 patients with CRC (81.8%) expressed positive results.

Conclusion Overexpression of the *CA9* gene in CRC of high sensitivity and specificity to be used as a tool to discriminate CRC from benign associate with high accuracy compare to *WT1* and *PRAME* genes.

Keywords

- ▶ colorectal cancer
- ▶ tumor-associated antigens
- ▶ real-time polymerase chain reaction
- ▶ CA9
- ▶ WT1
- ▶ PRAME

received
August 7, 2021
accepted after revision
October 25, 2021
published online
November 23, 2021

DOI <https://doi.org/10.1055/s-0041-1741323>.
ISSN 2237-9363.

© 2021. Sociedade Brasileira de Coloproctologia. All rights reserved.

This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Introduction

In 2018, more than 2 million new cases of colorectal cancer (CRC) were recorded worldwide, and caused around 1 million deaths that year.¹⁻⁴ This type of cancer ranks third in terms of incidence and second in terms of mortality.¹ Tumor-associated antigens (TAAs) are molecules and proteins expressed by tumor cells, and they may act as cancer markers.² In cases of CRC, TAAs are underexpressed in normal tissue but are overexpressed or mutated in tumor cells.

A member of the carbonic anhydrase family, *carbonic anhydrase IX (CA9)* is one of the genes that is overexpressed in tumor cells; it is a membrane protein that regulates cell proliferation in response to hypoxia.^{5,6} In 2006, Talvinen et al.⁷ found that *CA9* was the most up-regulated gene in cases of CRC through a complementary DNA (cDNA) microarray.

The *Wilms tumor (WT1)* gene is one of the genes involved in growth regulation and/or differentiation of cells.^{8,9} Previous studies have demonstrated that *WT1* is expressed in cancer cells derived from different types of cancers, including CRC,¹⁰ leukemia,¹¹ breast cancer,^{12,13} non-small-cell lung carcinoma (NSCLC),¹⁴ soft-tissue sarcoma (STS),¹⁵ and head and neck squamous cell carcinoma (HNSCC).¹⁶

The *preferentially expressed antigen in melanoma (PRAME)* gene is detected in melanoma, but its expression is absent or low in cases of CRC.¹⁷ The *PRAME* gene is expressed in many different cancers, and its expression correlates with prognosis and survival. It is expressed in NSCLCs, breast carcinomas, renal cell carcinomas (RCCs), head and neck cancers, Hodgkin lymphomas (HLs), sarcomas, Wilms tumors, and medulloblastomas.¹⁷

The present work was designed to assess the diagnostic and prognostic value of the *CA9*, *WT1*, and *PRAME* genes in cases of CRC.

Methods

Study Design and Setting

A prospective case-control study with CRC patients was conducted in the National Center of Cancer from March to December 2020. The following data were collected: age, gender, family history, tumor sites, tumor differentiation, and tumor stages. All patients were histologically diagnosed with CRC. The control group who referred to this center for screening purposes due to negative colonoscopies. Then all cases were submitted to molecular analysis.

Clinical Samples

Peripheral blood samples were collected from the 25 eligible patients newly-diagnosed with CRC in different stages, as well as from 10 patients in the control group. The samples had been sent to and preserved at the genetic laboratory of the National Center of Cancer. In total, 2 mL from each sample were put in ethylenediaminetetraacetic acid (EDTA) tubes. All samples were then submitted to DNA extraction and a

molecular study through real-time polymerase chain reaction (PCR).

Ethics Consideration

All patients signed the informed consent form for the present study, and approval was obtained from the Ethics in Research Committee of Dijlah University College.

DNA Extraction and Real-time PCR

The total genomic DNA of the samples from the CRC and control groups was extracted from the peripheral blood using the salting-out method,¹⁸ following the protocol provided by the manufacturer. Beta-catenin/Tcf is the constitutive gene, a double-stranded 15-nucleotide oligomer composed from first forward set contained *CCCT T TGATCT TACC*, and the second reverse set *CCCT T TGGCCT TACC*.¹⁹

These were analyzed using specific primers, which were designed using the Gene Runner software, version 4.0.9.68 Beta (►Table 1). The quantitative real-time PCR assay was performed in duplicate using the Applied Biosystems PCR Systems (Thermo Fisher Scientific, Waltham, MA, US). Synthesis of the suitable size PCR products were visualized by agarose gel electrophoresis. Genotyping was performed using the PCR-restriction fragment length polymorphism (PCR-RFLP) analytical method. The real-time PCR program is described and illustrated in ►Table 2 and ►Fig. 1 respectively.

Table 1 Sequences of the primers used in the present study

Primer		Sequence	Size (base pairs)
CA9	Forward	5'-GTGGAAGGCCACCGTTTC-3'	1,546
	Reversed	5'-CTCGTCAACTCTGGCAAAGG-3'	
WT1	Forward	5'-AGGCTTTGCTGCTGAGGAC-3'	1,962
	Reversed	5'-CAGGTCATGCATTCAAGCTG-3'	
PRAME	Forward	5'-CTTTCCTCGAAGGCCACCT-3'	1,479
	Reversed	5'-GTTATTGTGAGGACCTTAACGA-3'	

Table 2 Real-time polymerase chain reaction program

No	Steps	Temperature (C°)	Time	Cycles
I	Initial denaturation	50	2 minutes	1
II	Denaturation	95	10 minutes	1
III	Annealing	95	15 seconds	30
		65	60 seconds	
		72	60 seconds	
IV	Final extension	95	15 seconds	1

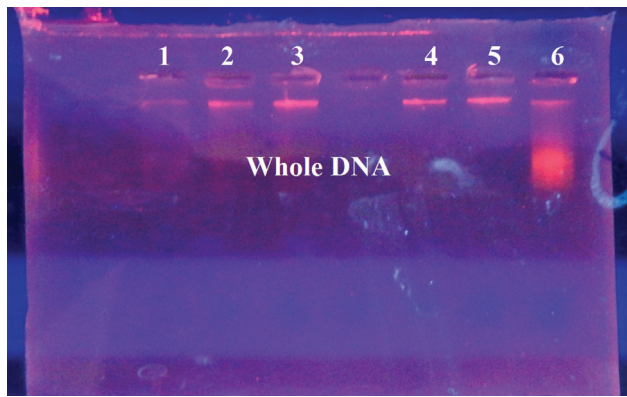


Fig. 1 Electrophoresis for the extraction of genomic DNA from samples in 1% agarose gel.

Statistical Methods

All data were analyzed using the Statistical Package for the Social Sciences (SPSS Statistics for Windows, IBM Corp., Armonk, NY, US) software. Frequency tables were analyzed using the Wilcoxon rank sum test to compare the two groups in a non-parametric pattern. The numerical variables were expressed as means and standard deviations (SDs). A two-sided p -value ≤ 0.05 was considered statistically significant.

Results

The CRC group consisted of 15 (60%) females and 10 (40%) male patients with mean age of 50.52 ± 9.8 years, while the control group included 4 (40%) female and 6 (60%) male patients with mean age of 47.7 ± 7.9 years. Only 2 (8%) patients in the CRC group and 1 (10%) individual in the control group had family history positive of CRC. The rectosigmoid was the most common site, reported in 13 (52%) patients, the rectum in 10 (40%) patients, and the anus and rectum in 2 (8%) individuals. Regarding tumor differentiation, it was classified as moderate (52%), well (44%), and poor (4%). Regarding the tumor, lymph node, and metastasis (TNM) staging, T2N0, and T3N0 were the most common classes, each observed in 6 (24%) patients. Metastasis were found in 4 (16%) patients, and lymphovascular and regional invasions were observed in 12 (48%) patients. Stage-II cancer was the most common, present in 12 (48%) patients, as shown in ► **Table 3**.

Out of 25 cases of CRC, 24 (96%) patients samples were CA9 positive, with strong statistically significant differences ($p < 0.00001$; sensitivity: 96%; specificity: 90%; positive predictive value [PPV]: 96%; negative predictive value [NPV]: 90%). Regarding the *WT1* gene, there were 44% of positive samples, with no statistically significant differences ($p = 0.055$; sensitivity: 44%; specificity: 90%; PPV: 91.67%; NPV: 39.13%). The *PRAME* gene was positive in 9 (36%) CRC patients, with no statistically significant differences ($p = 0.357$; sensitivity: 36%; specificity: 80%; PPV: 81.82%; NPV: 33.33%). As a result, the sensitivity and specificity of the CA9 gene were higher for the diagnosis and prognosis of CRC than the *WT1* and *PRAME* genes, with an accuracy reaching 94.29%, as shown in ► **Table 4**. ► **Table 5** shows a comparison

of the expression of TAAs of the *CA9*, *WT1*, and *PRAME* genes between the two study groups. Regarding the *CA9* gene, 24 (96%) patients in the CRC group and 1 (4%) individual in the control group expressed positive results, with strong statistically significant differences ($p < 0.0001$). s for the *WT1* gene, 11 (91.6%) CRC patients and 1 (8.4%) control expressed it, with strong statistically significant differences ($p < 0.0001$). In addition, 9 (81.8%) patients in the CRC group and 2 (18.2%) controls expressed the *PRAME* gene, with high statistically significant differences ($p < 0.001$). Gel electrophoresis of the PCR products of CRC patients were performed using the primers set for the *CA9*, *WT1*, and *PRAME* genes (forward and reversed) of DNA, as shown in ► **Fig. 2**.

Discussion

The incidence of CRC is increasing annually worldwide. The demographics of the CRC group were similar to those of the patients in most of the studies conducted in Iraq, such as those by Radhi et al.²⁰ in Al-Diwaniyah, by Alsafi et al.²¹ in Karbala, by Alshewered and Al-Naqqash² and by Alrubaia et al.³ in Baghdad, but differed from the demographics found in the studies by Alhilfi et al.⁴ in Misan, and by Khalil et al.²² in Duhok. In Western countries, 8% of the CRC cases are recorded in patients younger than 40 years of age, whereas in Asia and Africa the rates range between 15% and 35% for the same age group.²³⁻²⁵

Early detection of CRC can improve the chance of a successful management and better prognosis. Tumor-associated antigens and autoantibodies have generated increasing interest as biomarkers for the development of innovative diagnostic and prognostic tools that have an effective role in the management of human cancers.²⁶ Feitosa et al.²⁷ concluded that genetic counseling and proper screening programs are essential tools for the early diagnosis and follow-up of cases of CRC.

In the present study, CA9 expression showed high sensitivity and specificity for the detection of CRC, with high accuracy (of up to 94.29%). In fact, it can be used as a potential significant prognostic tool in CRC. These data implicate that the intensity of the CA9 expression seems to be a powerful independent prognostic factor, not confounded by the mode of treatment.

In a study, Korkeila et al.²⁹ found no correlation between the expression of CA9 and other clinical or histopathological factors of CRC, since most of their patients had received radiotherapy with or without chemotherapy before operation, which may have had an influence on tumor size, stage, grade, and nodal status. Driessen et al.³⁰ found a significant correlation between the *WT1* gene and the grade, stage, and lymph node status of different kinds of tumors, while Epping and Bernards¹⁷ demonstrated that the lymph node status of patients was not directly associated with the *PRAME* gene.

In their study, Oji et al.³¹ demonstrated that the *WT1* gene was overexpressed in the majority of the primary colorectal adenocarcinomas they examined, and it was the non-mutated, wild type. This result supported their hypothesis that the wild-type *WT1* gene does play an oncogenic role in the

Table 3 Clinical features of patients with colorectal adenocarcinoma (colorectal cancer group: patients 1-25; control group: patients 26-35)

ID	Age (years)	Gender	Family history	Tumor site	Differentiation	Tumor, Node, Metastasis classification	Stage
1	36	Female	Negative	Rectosigmoid	Well	T2N0	II
2	54	Female	Negative	Rectosigmoid	Moderate	T2N0	II
3	51	Male	Negative	Rectosigmoid	Well	T2N2	III
4	44	Female	Negative	Rectum	Moderate	T1N0	I
5	60	Male	Negative	Rectum	Well	T3N1M1	IV
6	37	Male	Negative	Rectosigmoid	Moderate	T3N0	II
7	29	Male	Negative	Rectosigmoid	Moderate	T3N1	III
8	53	Male	Negative	Rectum	Well	T2N0	II
9	63	Female	Negative	Rectum	Poor	T2N2M1	IV
10	56	Female	Negative	Rectum	Well	T3N0	II
11	39	Female	Positive	Rectosigmoid	Well	T3N1	III
12	55	Female	Negative	Rectum	Moderate	T3N0	II
13	40	Male	Negative	Rectosigmoid	Well	T2N2	III
14	54	Female	Negative	Rectosigmoid	Moderate	T2N0	II
15	52	Female	Negative	Rectosigmoid	Moderate	T3N1	III
16	70	Male	Negative	Rectosigmoid	Well	T3N0	II
17	62	Female	Negative	Rectosigmoid	Moderate	T2N1M1	IV
18	61	Female	Negative	Anus and rectum	Moderate	T2N0	II
19	55	Male	Negative	Rectum	Moderate	T2N0	II
20	59	Male	Negative	Rectosigmoid	Moderate	T3N2M1	IV
21	48	Female	Negative	Anus and rectum	Moderate	T3N0	II
22	47	Male	Positive	Rectosigmoid	Moderate	T2N1	III
23	47	Female	Negative	Rectum	Well	T1N1	III
24	50	Female	Negative	Rectum	Well	T3N0	II
25	41	Female	Negative	Rectum	Well	T3N1	III
26	39	Male	Negative				
27	50	Male	Negative				
28	44	Male	Negative				
29	43	Female	Negative				
30	60	Male	Negative				
31	52	Female	Negative				
32	56	Female	Negative				
33	55	Male	Negative				
34	38	Female	Positive				
35	40	Male	Negative				

tumorigenesis of CRC. The results of the present study are in line with those of the study by Oji et al.³¹ since the *WT1* was expressed in 44% of the CRC group, but without any statistical significance. Moreover, Oji et al.³¹ reported that the over-expression was observed in most of the patients examined by them, and this high frequency might indicate that the *WT1* gene is one of the critical genetic events in tumorigenesis.

They reported that the analysis of the correlation between the expression of *WT1* in colorectal hyperplastic mucosa, adenoma, and carcinoma, is of interest and importance.³¹

Many previous studies³²⁻³⁴ indicated that the expression of the *PRAME* gene is not significantly associated with relapse-free survival, tumor grade and size, and lymph node status in cases of CRC. These results may be due to

Table 4 Gene expression results

Tumor-associated antigens		Colorectal cancer group (n = 25)	Control group (n = 10)	p-value
		N (%)		
CA9 [§]	Positive	24 (96)	1 (10)	< 0.00001
	Negative	1 (4)	9 (90)	
WT1 [*]	Positive	11 (44)	1 (10)	0.055
	Negative	14 (56)	9 (90)	
PRAME [#]	Positive	9 (36)	2 (20)	0.357
	Negative	16 (64)	8 (80)	

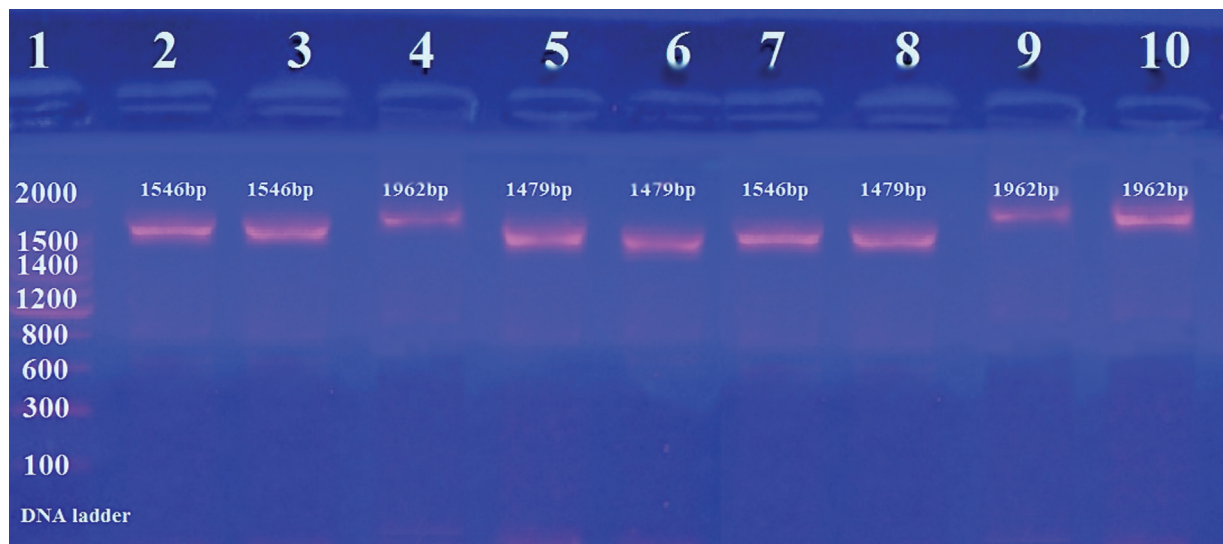
Notes: [§]Sensitivity: 96%; specificity: 90%; positive predictive value (PPV): 96%; negative predictive value (NPV): 90%; accuracy: 94.29%.

^{*}Sensitivity: 44%; specificity: 90%; PPV: 91.67%; NPV: 39.13%; accuracy: 57.14%.

[#]Sensitivity: 36%; specificity: 80%; PPV: 81.82%; NPV: 33.33%; accuracy: 48.57%.

Table 5 Comparison of the expression of tumor-associated antigens of the CA9, WT1, and PRAME genes between the two study groups

Tumor-associated antigens	Colorectal cancer group	Control group	p-value
	N (%)		
CA9	24 (96)	1 (4)	< 0.0001
WT1	11 (91.6)	1 (8.4)	< 0.0001
PRAME	9 (81.8)	2 (18.2)	< 0.001

**Fig. 2** Gel electrophoresis of amplified DNA from CRC patients by using the primers set CA9, WT1, and PRAME (forward and reversed). Lane 1: DNA ladder. Lanes 2, 3, 7: CRC DNA positive for 1,546 base pairs (bp) (CA9). Lanes 4, 9, 10: CRC DNA positive for 1,962 bp (PRAME). Lanes 5, 6, 8: CRC DNA positive for 1,479 bp (WT1).

the fact that the exact function of the PRAME gene in the tumorigenesis of CRC remains controversial.³⁴

The analysis of a very small sample of patients (25 patients with CRC and 10 controls) is a main limitation of the present study; further research with larger samples should be considered to confirm the results found.

Conclusion

The overexpression of the CA9 gene in CRC of high sensitivity and specificity to be used as a tool for discriminating malignant colorectal tumors from benign since with high accuracy compare with the WT1 and PRAME genes. The diagnostic and prognostic indications of CA9 are more implications to

expressed CRC than that of WT1 and PRAME. Furthermore, studies on the molecular mechanisms of the WT1 and PRAME genes are mandatory to provide new information about the function and regulating pathways of these two genes in the tumorigenesis of CRC, since they are expressed, but with low levels of significance. Further research with larger samples should be considered to confirm the results found in the present study. To our knowledge, the present is the first study to report on the importance of the CA9, WT1, and PRAME genes in cases of CRC.

Financial support

The authors have no sources of funding to declare.

Conflict of Interests

The authors have no conflict of interests to declare.

References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68(06):394–424
- Alshewered AS, Al-Naqqash MA. Rectal cancer and chemoradiation in Iraq: systematic review and meta-analysis. *J Coloproctol (Rio J)* 2019;39:309–318
- Alrubaia AMM, Al-Naqqash MA, Alshewered AS. Epidemiological, and prognostic single-center study of anal carcinoma. *J Coloproctol (Rio J)* 2020;40(03):202–208
- Alhilfi HSQ, Almohammadawi KOM, Alsaad RKA, et al. Colorectal cancer epidemiology and clinical study in Misan. *J Coloproctol (Rio J)* 2019;39(02):159–162
- Thiry A, Dogné JM, Masereel B, Supuran CT. Targeting tumor-associated carbonic anhydrase IX in cancer therapy. *Trends Pharmacol Sci* 2006;27(11):566–573
- Wykoff CC, Beasley NJ, Watson PH, et al. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res* 2000;60(24):7075–7083
- Talvinen K, Tuikkala J, Grönroos J, et al. Biochemical and clinical approaches in evaluating the prognosis of colon cancer. *Anticancer Res* 2006;26(6C):4745–4751
- Stauss HJ, Thomas S, Cesco-Gaspere M, et al. WT1-specific T cell receptor gene therapy: improving TCR function in transduced T cells. *Blood Cells Mol Dis* 2008;40(01):113–116
- Algar E. A review of the Wilms' tumor 1 gene (WT1) and its role in hematopoiesis and leukemia. *J Hematother Stem Cell Res* 2002;11(04):589–599
- Oji Y, Ogawa H, Tamaki H, et al. Expression of the Wilms' tumor gene WT1 in solid tumors and its involvement in tumor cell growth. *Jpn J Cancer Res* 1999;90(02):194–204
- Inoue K, Sugiyama H, Ogawa H, et al. WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. *Blood* 1994;84(09):3071–3079
- Loeb DM, Evron E, Patel CB, et al. Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. *Cancer Res* 2001;61(03):921–925
- Miyoshi Y, Ando A, Egawa C, et al. High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. *Clin Cancer Res* 2002;8(05):1167–1171
- Oji Y, Miyoshi S, Maeda H, et al. Overexpression of the Wilms' tumor gene WT1 in de novo lung cancers. *Int J Cancer* 2002;100(03):297–303
- Ueda T, Oji Y, Naka N, et al. Overexpression of the Wilms' tumor gene WT1 in human bone and soft-tissue sarcomas. *Cancer Sci* 2003;94(03):271–276
- Oji Y, Inohara H, Nakazawa M, et al. Overexpression of the Wilms' tumor gene WT1 in head and neck squamous cell carcinoma. *Cancer Sci* 2003;94(06):523–529
- Epping MT, Bernards R. A causal role for the human tumor antigen preferentially expressed antigen of melanoma in cancer. *Cancer Res* 2006;66(22):10639–10642
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16(03):1215
- Korinek V, Barker N, Morin PJ, et al. Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. *Science* 1997;275(5307):1784–1787
- Radhi AA, Muslim OT, AbdImaged MA. Epidemiological distribution of colorectal cancer in AL-Diwaniyah province, Iraq: an observational study. *J Pharm Sci & Res.* 2018;10(07):1758–1760
- Alsafi RAR, Metib NJ, Hameedi AD, et al. The Clinical and Pathological Characteristics of Colorectal Cancer in Young Age Group in Karbala Province/ Iraq. *Karbala J Med.* 2018;11(02):4025–4031
- Khalil KH, Al-Hassawi BA, Abdo JM. Histopathological evaluation of colorectal carcinoma. *Duhok Medical Journal.* 2018;12(02):45–68
- Al Dahhan SA, Al Lami FH. Epidemiology of Colorectal Cancer in Iraq, 2002–2014. *Gulf J Oncolog* 2018;1(26):23–26
- <https://www.nice.org.uk/> The National Institute for Health and Care Excellence/guidance/cg131/ colorectal cancer diagnosis and management/ 2014
- <http://publications.iarc.fr/> The International Agency for Research on Cancer/Non-Series-Publications/World cancer report/2014
- Wang MJ, Ping J, Li Y, et al. The prognostic factors and multiple biomarkers in young patients with colorectal cancer. *Sci Rep* 2015;5:10645
- Feitosa MR, de Oliveira THGF, Kondo BRP, et al. The epidemiological and clinical features of familial adenomatous polyposis in Ribeirão Preto. *J Coloproctol (Rio J)* 2013;33(03):126–130
- Giatromanolaki A, Koukourakis MI, Sivridis E, et al. Expression of hypoxia-inducible carbonic anhydrase-9 relates to angiogenic pathways and independently to poor outcome in non-small cell lung cancer. *Cancer Res* 2001;61(21):7992–7998
- Korkeila E, Talvinen K, Jaakkola PM, et al. Expression of carbonic anhydrase IX suggests poor outcome in rectal cancer. *Br J Cancer* 2009;100(06):874–880
- Driessen A, Landuyt W, Pastorekova S, et al. Expression of carbonic anhydrase IX (CA IX), a hypoxia-related protein, rather than vascular-endothelial growth factor (VEGF), a pro-angiogenic factor, correlates with an extremely poor prognosis in esophageal and gastric adenocarcinomas. *Ann Surg* 2006;243(03):334–340
- Oji Y, Yamamoto H, Nomura M, et al. Overexpression of the Wilms' tumor gene WT1 in colorectal adenocarcinoma. *Cancer Sci* 2003;94(08):712–717
- Oberthuer A, Hero B, Spitz R, Berthold F, Fischer M. The tumor-associated antigen PRAME is universally expressed in high-stage neuroblastoma and associated with poor outcome. *Clin Cancer Res* 2004;10(13):4307–4313
- Huang Q, Li L, Lin Z, et al. Identification of Preferentially Expressed Antigen of Melanoma as a Potential Tumor Suppressor in Lung Adenocarcinoma. *Med Sci Monit* 2016;22(22):1837–1842
- Wadelin F, Fulton J, McEwan PA, Spriggs KA, Emsley J, Heery DM. Leucine-rich repeat protein PRAME: expression, potential functions and clinical implications for leukaemia. *Mol Cancer* 2010;9:226