Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of the diagnostic performance of serum P53 protein for the diagnosis of colorectal cancer.

Reham Abdel-Aziz[a], Mohamed M. Omran[b*], Abdelfattah M. Attallah[a], Mohamed El-Far[c*]

[a] *Research and development department , Biotechnology Research Center, New Damietta, Egypt*

[b] *Chemistry department, Faculty of Science, Helwan University, Cairo, Egypt*

[c] *Chemistry department, Faculty of Science, Mansoura University, Mansoura, Egypt*

Abstract

Early colorectal cancer (CRC) diagnosis improves disease prognosis and treatment. However, current approaches are suboptimal, and no serum-based test is sufficient for widespread use. This study aimed to evaluate serum P53 protein efficacy as a non-invasive CRC marker. Serum P53 protein level was evaluated in 237 participants (127 CRC and 70 benign disorder patients and 40 healthy controls) using western blotting and ELISA. Area under receiver operating characteristic curve (AUC) was applied for evaluation diagnostic performance. An immunoreactive band at 53-KDa was detected corresponding to Serum P53 protein in only patients with colorectal diseases. Aberrant P53 both detection rates and optical density level in patients with CRC (69.3%; 1.19±0.03) were significantly (P=0.001) higher than patients with non-malignant benign growth (27.1%; 0.51±0.02). Serum P53 protein effectively identified CRC (AUC=0.90, 87.0% sensitivity and 76.4% specificity) from all noncancerous individuals and tumor early stages (AUC=0.84, 85.5% sensitivity and 72.9% specificity) from benign disorders. Elevated detection rates and optical density levels were significantly associated with tumor advanced stages (P=0.015), high grades (P=0.001), lymph node invasion (P=0.010) and distant metastasis (P=0.001). In conclusion, Serum P53 protein could be an effective CRC biomarker especially for differentiating early disease stages from benign disorders.

Keywords: Colorectal cancer; Biomarkers; Serum P53 protein; Early diagnosis; Tumor severity; Egyptian patients

Introduction

With over one million new cases per year, colorectal cancer (CRC) is one of the most common cancers worldwide [1]. In Egypt, CRC is the six cancers in both males and females [2]". The incidence rate of CRC is 5.1% in males and 4.7% in females [3, 4]. Despite improvement in its management through immunotherapy, radiotherapy, chemotherapy and surgery, CRC is still a leading cause of death worldwide [5]. The prognosis for CRC patients is associated with disease pathological stage and the majority of CRC has been shown as potentially curable and preventable by early detection and early-stage tumors removal [6,7]. Thus, early detection is crucial to decrease incidence and mortality related to CRC [8].

Colonoscopy, the gold-standard test, allows for both detection and removal of polyps and cancers during the same procedure. Despite its advantages, it is an invasive not suitable test (significant bleeding can occur in up to 8.7/1,000), need for more thorough bowel sedation and cleansing and need to stop certain medications, especially blood thinners such as clopidogrel, warfarin or aspirin [9]. Therefore, search of inexpensive, non-invasive and convenient biomarker with a high degree of efficacy, is an effective to monitor CRC [5]. Biomarkers are a key tool in prognostication, early detection, predicting treatment response and survival [5]. Many classical markers have been used in CRC detection, but carcinoembryonic antigen (CEA) and carbohydrate antigen (CA 19.9) are the most common. However, they are not suitable for the early CRC detection owing to the low specificity and sensitivity[6]. Therefore, further tools are required to support CRC early identification [5]. In human cancers, one of the most studied tumor suppressor genes is *p53* and its protein executes in tumor cells the function of cell cycle arrest, pro-apoptosis, and DNA reparation [10]. By immunohistochemistry, P53 protein was detected in high percentage of colorectal adenocarcinomas[11]. Positive tumors were more

significantly frequent in the distal colon, and demonstrated high cell proliferation rate[11]. Moreover, CRC patients with abnormal P53 were at increased death risk[12]. But, P53 diagnostic value in CRC remains unclear. Therefore, this study to evaluate serum P53 protein clinical relevance and to analyses its association with some CRC related pathological factors.

1. Material and methods
Clinical samples

Serum samples from 127 CRC patients with a primary tumor (73 males and 54 females; mean age of 55.6±10.9 years) and 70 patients with benign growth (39 males and 31 females; mean age of

50.5±10.5 years) were provided by Oncology center, Mansoura University Hospitals, Mansoura, Egypt between June 2020, and February 2021. They were classified according to colonoscopy. In addition, 40 age- and sex-matched healthy individuals (22 males and 18 females; mean age of 47.6±12.7 years) were included. Tumor features were registered according to the Union for International Contrele Cancer-Tumor-Node-Metastasis (TNM) Staging System[9]. From each participant, an informed consent was gotten. This study was approved by ethics and scientific-committees of Mansoura University Hospitals, Mansoura, Egypt.

Table 1. Patient's characteristics

Variables	Normal	Benign	Cancer	P value	
				All groups	Cancer *vs* benign
Age (years)	47.6± 12.7	50.5±10.5	55.6±10.9	0.064	0.095
Gender (male/female)	22/18	39/31	73/54	0.123	0.159
AST (U/L)	21.8±5.2	23.8±6.2	31.9±14.1	0.001	0.088
ALT (U/L)	21.3±3.9	24.4±4.7	30.1±15.2	0.001	0.275
ALP (U/L)	63.1±15.6	152.9±26.4	222.1±31.7	0.0001	0.436
Hemoglobin (g/dL)	13.5±1.5	11.2±1.4	9.9±1.6	0.001	0.019
RBCs (x10^{12}/L)	4.9±0.5	4.3±0.6	3.9±0.5	0.001	0.047
WBCs (x10^9/L)	5.2±1.2	8.8±5.4	12.3±3.6	0.001	0.020
Neutrophils (x10^9/L)	3.3±1.6	7.8±2.1	11.1±4.9	0.0001	0.036
Lymphocytes (x10^9/L)	2.2±0.8	1.6±0.6	1.2±0.4	0.0001	0.011
Platelets count (x10^9/L)	330.1±98.9	280.2±48.7	215.3±75.7	0.001	0.028
CEA (U/L)	3.1±0.7	32.8±9.6	178.3±36.1	0.001	0.001
CA19.9 (U/L)	15.2±4.3	45.5±2.7	98.2±26.1	0.001	0.007
Tumor stage (early T≤2/late T>2)	–	–	58/59	–	–
Tumor grade (low G≤2/high G>2)	–	–	73/54	–	–
Lymph node (-ve/+ve)	–	–	79/48	–	–
Distant metastasis (-ve/+ve)	–	–	90/37	–	–

Laboratory tests

After centrifugation of blood samples, at 4,000 rpm for 15 minutes, the serum was stored at −20 °C until used. Serum samples were used for routine tests analysis including liver enzymes and alkaline phosphatase using biochemistry analyzer (Biosystems S.A., Barcelona, Spain). According to the industrial prescript, carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9) were measured by commercial ELISA assay (IRMA) (IBL, Germany). Blood samples treated with an anticoagulant (KEDTA) were used for a complete blood count (Hematology analyzer, Sysmex, Japan).

Detection of P53 in colorectal cancer

As previously described by Attallah et al [7]. Serum P53 protein was detected in patients with colorectal cancer compared to patients with benign

inflammations and healthy controls using western blotting and ELISA techniques using monospecific rabbit antibodies (ABC Diagnostics, New Damietta, Egypt). The optical density was reading using microplate spectrophotometer (Metreiteck, Axiom, Burstadt, Germany) at 405 nm. The cutoff for ELISA technique is calculating as the mean optical density (OD) for 16 serum of normal people ± 3SD was equal 0.28, and serum samples from 20 colon cancer patients showed concentration above the cut off level

Statistical analysis

Data were statistically analyzed using SPSS (version 20) and Graph Pad Prism (version 7) programs. Different parameters have been represented as mean ± standard deviation (SD). Categorical variables were expressed as absolute numbers or percentages. Significant differences between groups were performed based on Chi-squared (*X2) or ANOVA* tests, appropriately, followed

by Fisher's Least as post hoc test. *P* value considered statistically significant at a two sided <0.05. Correlation between P53 optical density levels and age, clinical parameters and tumor markers was assessed by Pearson correlation analysis**.** To determine serum P53 protein diagnostic power, receiver operating characteristic (ROC) curve was used.

Results
Identification of P53 in sera of colorectal patients

There was no significant difference ($p > 0.05$) in mean age and gender among three groups of subjects as shown in Table1. Compared to patients with benign disorders and healthy controls, the 127 CRC patients were associated with elevated liver enzymes and white blood cells and decreased hemoglobin, red blood cells and platelets count (Table 1). Also, cancer patients were presented according to tumor markers levels (CEA and CA 19.9), tumor stage, tumor grades, lymph node invasion, and distant organ metastasis (Table 1). By western blotting, the presence of serum P53, at 53 KDa, expression in CRC was confirmed (Figure 1A).

Detection of P53 using ELISA

All participants of each group were screened for circulating P53 protein. Positivity for serum P53 protein was found in 88/127 (69.3%) patients with CRC in contrast to 19/70 (27.1%) in non-malignant benign growth and 0/40 (0%) in healthy controls (Table 2). Also, elevated detection rates were significantly associated with tumor advanced stages (P=0.002), high grades (P=0.003), lymph node invasion (P=0.0001) and distant metastasis (P=0.010) (Table 2).

Diagnostic power of P53 in colorectal cancer

Quantitatively, the distribution of serum P53 protein optical density levels in patients with colorectal disorders was presented in Figure 1B. In general, CRC patients (1.19±0.03) were associated with higher serum P53 protein levels than patients with benign (0.51±0.02) disorders (P=0.001). Elevated P53 levels were also significantly associated with tumor severity features including, late stages (1.35±0.07), high histological grades (1.72±0.09), lymph node invasion (1.24±0.08), and distant organ metastasis (Figure 2).

Based on ROC analysis serum P53 protein yielded a good diagnostic performance to distinguish CRC (AUC=0.90, 87.0% sensitivity and 76.4% specificity) from all non-cancerous individual (Figure 3A). This valuable power for CRC identification did not significantly affect (AUC=0.87, 86.2% sensitivity and 72.9% specificity) when compared with benign disorders only (Figure 3B). Concerning early

detection, serum P53 protein had a good diagnostic power in distinguishing CRC patients with early stages from those with benign growth (AUC=0.84, 85.5% sensitivity and 72.9% specificity) (Figure 3C). In contrast to patient's age, Pearson's correlation analysis revealed that there was significantly (P<0.05) positive correlation between P53 and white blood cells, CEA and CA19.9 levels and negative correlation with hemoglobin, red blood cells and platelet count (Table 3).
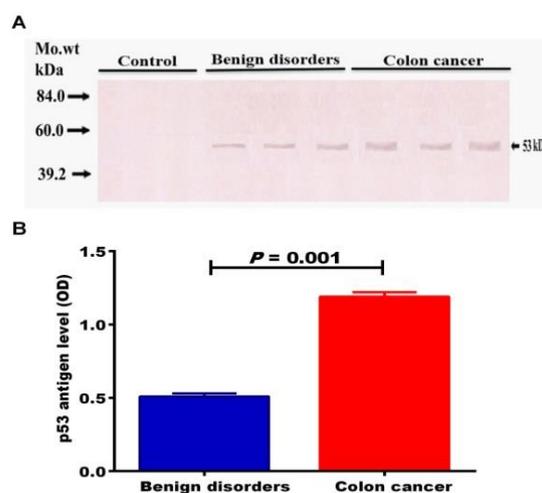

FIGURE (1): The identification and quantification of serum p53 protein in CRC (A) Serum P53 protein identification using western blotting analysis. Molecular weight markers were bovine serum albumin, ovalbumin and carbonic anhydrase corresponding to 84.0, 60.0 and 39.2 kDa, respectively, (B) Optical density level of P53 in colorectal cancer compared to patients with benign disorders.

P53 to discriminate colorectal cancer patients from (A) all non-cancerous individuals and (B) patients with benign growth and (C) between patients with tumor early stages and those with benign growth. AUC: area under curve, Sn: sensitivity, Sp: specificity.

Discussion

Despite that its incidence rate is increasing globally especially in highly developed countries, CRC is also increasing, due to westernization, in middle- and low-income countries [14]. In contrast to late disease stages, CRC diagnosis at earlier/middle stages has a better prognosis [15]. Moreover, there has been limited clinical success in developing non-invasive, effective, diagnostic techniques for early detection of CRC [17].

In this study using respective mono-specific antibodies, a single immunoreactive band

corresponding to serum P53 protein was shown at 53-kDa. This is the known molecular weight of the cellular phosphoprotein P53 that was previously reported by varied studies [7, 15]. Accumulating mutant proteins from malignant cells can be released into serum and, therefore, these proteins could be easily detected by ELISA to a large degree in different laboratories [7]. In this study using ELISA, aberrant P53 both detection rates and optical density level in patients with CRC (69.3%; 1.19±0.03) were significantly (*P*=0.001) higher than patients with non-malignant benign growth (27.1%; 0.51±0.02).

As found in about 50% of sporadic tumors, mutation of the P53 tumor suppressor is a central driver of CRC carcinogenesis [18]. Beside this mutation, abnormalities of P53 gene expression and protein are common in CRC [19]. Different studies that have examined both P53 mutation and expression data for CRC have reported an agreement of mutation and immunohistochemistry detection between 76 and 53% [18, 20]. In the classic adenoma-to-carcinoma sequence of CRC tumor genesis, these P53 aberrations are considered a late event that is associated with adenoma transition to carcinoma [21].

P53 aberrations significance as a prognostic CRC marker remains a matter of controversy [18]. Here, elevated detection rates and optical density levels were significantly associated with tumor advanced stages (*P*=0.015), high grades (*P*=0.001), lymph node invasion (*P*=0.010) and distant metastasis (*P*=0.001). It was suggested that P53 aberrations frequency increases with tumor stage in CRC [18]. Many investigations, each included at least 100 CRC patients, reported that overexpression of P53 protein has been associated with worse CRC outcomes in multivariate or univariate survival analyses [22-24]. Other studies reported the opposite result [24, 25]; or have found no association [27]. However, Munro et al. performed systematic review concerning P53 expression data (included data from 12,257 patients) across all tumor stages and they concluded that CRC patients with abnormal P53 were at increased death risk [28].

Our findings showed that, serum P53 protein could be effective in CRC identification (AUC=0.90, 87.0% sensitivity and 76.4% specificity) from all noncancerous individuals. This valuable power for CRC identification did not significantly affect (AUC=0.87, 86.2% sensitivity and 72.9% specificity) when compared with benign disorders only Interestingly, these diagnostic performances are well comparable to other CRC established biomarkers in differentiating CRC from healthy individuals such as CEA (AUC=0.790, 65.0% sensitivity, 90.7% specificity), CA 19-9 (AUC=0.739, 62.0% sensitivity, 89.0% specificity) and CA 72-4 (AUC=0.746, 45.0% sensitivity, 96.0% specificity) [29]". The specificity

of these markers significantly affected when compared with patients with benign disorders [30].

Table 2. Detection of P53 using ELISA

Categories	Phosphoprotein P53		*P* value
	-ve (%)	+ve (%)	
Groups			
Healthy	40 (100.0)	0 (0.0)	
Benign	51 (72.9)	19 (27.1)	0.0001
Colorectal cancer	39 (30.7)	88 (69.3)	
Tumor stages			
Early (≤T2)	26 (44.8)	32 (55.2)	0.002
Late (>T2)	13 (18.8)	56 (81.2)	
Tumor grades			
Low ≤G2	30 (41.1)	43 (58.9)	0.003
High >G2	9 (16.7)	45 (83.3)	
Lymph node involvement			
Absent (n=79)	36 (45.6)	43 (54.4)	0.0001
Present (n=48)	3 (6.3)	45 (93.7)	
Distant organ metastasis			
Absent (n=90)	34 (37.8)	56 (62.2)	0.01
Present (n=37)	5 (13.5)	32 (86.5)	

Table 3. Correlation of P53 with age and clinical data of colorectal cancer patients

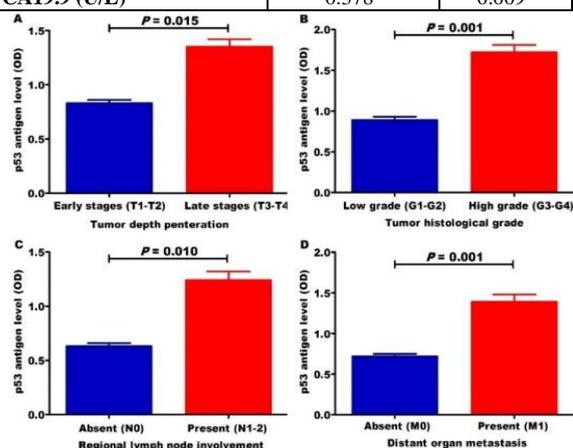| Variables	Correlation coefficient (*r*)	*P* value
Age (years)	0.138	0.423
Hemoglobin (g/dL)	-0.329	0.015
RBCs (x1012/L)	-0.414	0.0001
WBCs (x109/L)	0.240	0.029
Neutrophils (x109/L)	0.360	0.004
Lymphocytes (x109/L)	-0.396	0.0005
Platelets count (x109/L)	-0.355	0.005
CEA (U/L)	0.490	0.0001
CA19.9 (U/L)	0.378	0.009

FIGURE (2): Distribution of P53 optical density level according to tumor (A) stages, (B) grades, (C) lymph node invasion and (D) distant metastasis.
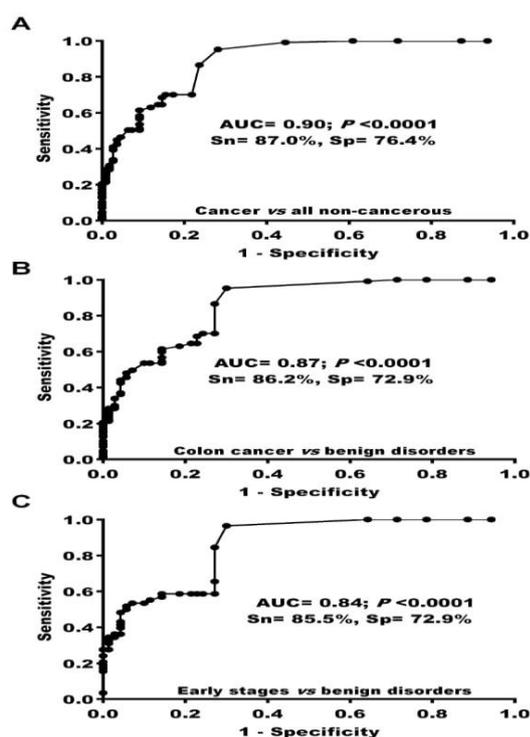
Figure 3. Receiver operating characteristic curve of P53 to discriminate colorectal cancer patients from (A) all non-cancerous individuals and (B) patients with benign growth and (C) between patients with tumor early stages and those with benign growth. AUC: area under curve, Sn: sensitivity, Sp: specificity.

Serum P53 protein had a good diagnostic power in distinguishing CRC patients with early stages from those with benign growth (AUC=0.84, 85.5% sensitivity and 72.9% specificity) and it was significantly correlated with CEA and CA19.9. This sensitivity is superior to that of CEA (43-69%), CA72-4 (9-31%), CA 19-9 (18-65%), CA125 (57.1%), vascular endothelial growth factor (35%-86%) and interleukin 3 (55%) in detecting CRC early stages [31-33].

This work was associated with some limitations including small sample volume and quantification of serum p53 level (ng/mL). Therefore further multicenter and large scale studies are needed to evaluate this potential clinical usefulness and to evaluate the value of P53 combination with other CRC established markers for CRC patients monitoring and to assess its role during course of therapy.

Conclusion

In conclusion, P53 can be a useful indicator for CRC clinical assessment. It is superior to other CRC biomarkers including CEA and CA 19-9 for CRC diagnosis owing to its higher sensitivity and specificity particularly in early stages.

References
[1]   Siegel, R. L.; Miller, K. D.; Goding Sauer, A.; Fedewa, S. A.; Butterly, L. F.; Anderson, J. C.; Cercek, A.; Smith, R. A.; Jemal, A. Colorectal cancer statistics, 2020. *CA Cancer J Clin*. 2020, *70*(3), 145-164.

[2]   Suliman, A. Marwa.; Zamzam, L. Maha.; Omar, T. Ayman.; Fahmy, N. Nermin . Clinicopathological Profile of Colorectal Cancer Patients in Suez Canal University Hospitals-Egypt. *Journal of Cancer Biology & Research*.2020, *8*(1), 1127-1131.

[3]   Bader El Din, G. Noha.; Ibrahim, K. Marwa.;El-Shenawy, Reem.; Salum, M. Ghada.; Farouk,Sally.; Zayed, Naglaa.; Khairy, Ahmed.; El Awady, Mostafa. MicroRNAs expression profiling in Egyptian colorectal cancer patients. *IUBMB*. 2020, *72*(2), 275-284.

[4]   Jasim, A. Saade; Abdulameer, H. Fadhaa; Mikhlef, A. Bilal; Mutar, A. Ayad. The Role of TROP2 Expression on Progression of Colon Cancer in Iraqi Patients. *Egyptian Journal of Chemistry*. 2022, 65(6):189-192

[5]   Ogunwobi, O. O.; Mahmood, F.; Akingboye, A. Biomarkers in Colorectal Cancer: Current Research and Future Prospects. *International journal of molecular sciences*. 2020, *21*(15), 5311.

[6]   Jelski, W.; Mroczko, B. Biochemical Markers of Colorectal Cancer - Present and Future. *Cancer management and research*. 2020, *12*, 4789-4797.

[7]   Gao, Y.; Wang, J.; Zhou, Y.; Sheng, S.; Qian, S. Y.; Huo, X. Evaluation of Serum CEA, CA19-9, CA72-4, CA125 and Ferritin as Diagnostic Markers and Factors of Clinical Parameters for Colorectal Cancer. *Sci Rep*. 2018, *8*(1), 2732.

[8]   Gaber, O. Moustafa. Therapeutic Potentials of Cyclic Peptides as Promising Anticancer Drugs. *The Egyptian Cancer of Chemistry*.2021, 64(4):1777-1787.

[9]   Issa, I. A.; Noureddine, M. Colorectal cancer screening: An updated review of the available options. *World journal of gastroenterology*. 2017, *23*(28), 5086-5096.

[10] Attallah, A. M.; El-Far, M.; Abdelrazek, M. A.; Omran, M. M.; Attallah, A. A.; Elkhouly, A. A.; Elkenawy, H. M.; Farid, K. Combined use of nuclear phosphoprotein c-Myc and cellular

_____

phosphoprotein p53 for hepatocellular carcinoma detection in high-risk chronic hepatitis C patients. *Br J Biomed Sci*. 2017, *74*(4), 170-175.

[11] Scott, N.; Sagar, P.; Stewart, J.; Blair, G. E.; Dixon, M. F.; Quirke, P. p53 in colorectal cancer: clinicopathological correlation and prognostic significance. *Br J Cancer*. 1991, *63*(2), 317-319.

[12] Smith, F. M.; Stephens, R. B.; Kennedy, M. J.; Reynolds, J. V. P53 abnormalities and outcomes in colorectal cancer: a systematic review. *Br J Cancer*. 2005, *92*(9), 1813.

[13]   Amin, B. Mahul.; Greene, L. Frederick.; Edge, B. Stephen.; Compton, C .Carolyn .;Gershenwald, E. Jeffrey.; Brookland, K. Robert.; Meyer, Laura.; Gress, M. Donna.; Byrd, R. David.;Winchester ,P. David. The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA. 2017, 67(2)93-99.

[14] Xi, Y.; Xu, P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol.* 2021, *14*(10), 101174.

[15] Feng, R. M.; Zong, Y. N.; Cao, S. M.; Xu, R. H. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer Commun (Lond)*. 2019, *39*(1), 22.

[16] Luo, H.; Zhao, Q.; Wei, W.; Zheng, L.; Yi, S.; Li, G.; Wang, W.; Sheng, H.; Pu, H.; Mo, H.; Zuo, Z.; Liu, Z.; Li, C.; Xie, C.; Zeng, Z.; Li, W.; Hao, X.; Liu, Y.; Cao, S.; Liu, W.; Gibson, S.; Zhang, K.; Xu, G.; Xu, R. H. Circulating tumor DNA methylation profiles enable early diagnosis, prognosis prediction, and screening for colorectal cancer. *Sci Transl Med*.2020, *12*(524).

[17] Wei, H.; Qu, L.; Dai, S.; Li, Y.; Wang, H.; Feng, Y.; Chen, X.; Jiang, L.; Guo, M.; Li, J.; Chen, Z.; Chen, L.; Zhang, Y.; Chen, Y. Structural insight into the molecular mechanism of p53-mediated mitochondrial apoptosis. *Nat Commun*. 2021, *12*(1), 2280.

[18] Williams, D. S.; Mouradov, D.; Browne, C.; Palmieri, M.; Elliott, M. J.; Nightingale, R.; Fang, C. G.; Li, R.; Mariadason, J. M.; Faragher, I.; Jones, I. T.; Churilov, L.; Tebbutt, N. C.; Gibbs, P.; Sieber, O. M. Overexpression of TP53 protein is associated with the lack of adjuvant chemotherapy benefit in patients with stage III colorectal cancer. *Mod Pathol*. 2020, *33*(3), 483-495.

[19] Leahy, D. T.; Salman, R.; Mulcahy, H.; Sheahan, K.; O'Donoghue, D. P.; Parfrey, N. A. Prognostic significance of p53 abnormalities in colorectal carcinoma detected by PCR-SSCP and immunohistochemical analysis. *J Pathol*. 1996, *180*(4), 364-370.

[20] Smith, D. R.; Ji, C. Y.; Goh, H. S. Prognostic significance of p53 overexpression and mutation in colorectal adenocarcinomas. *Br J Cancer*. 1996, *74*(2), 216-223.

[21] Fearon, E. R.; Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell*. 1990, *61*(5), 759-767.

[22] Chen, J.; Tang, H.; Wu, Z.; Zhou, C.; Jiang, T.; Xue, Y.; Huang, G.; Yan, D.; Peng, Z. Overexpression of RBBP6, alone or combined with mutant TP53, is predictive of poor prognosis in colon cancer. *PLoS One*. 2013, *8*(6), e66524.

[23] Zhang, M.; Cui, F.; Lu, S.; Lu, H.; Jiang, T.; Chen, J.; Zhang, X.; Jin, Y.; Peng, Z.; Tang, H. Increased expression of prothymosin-α, independently or combined with TP53, correlates with poor prognosis in colorectal cancer. *Int J Clin Exp Pathol*. 2014, *7*(8), 4867-4876.

[24] Ji, L.; Wei, Y.; Jiang, T.; Wang, S. Correlation of Nrf2, NQO1, MRP1, cmyc and p53 in colorectal cancer and their relationships to clinicopathologic features and survival. *Int J Clin Exp Pathol*. 2014, *7*(3), 1124-1131.

[25] Adrover, E.; Maestro, M. L.; Sanz-Casla, M. T.; del Barco, V.; Cerdán, J.; Fernández, C.; Balibrea, J. L. Expression of high p53 levels in colorectal cancer: a favourable prognostic factor. *Br J Cancer*. 1999, *81*(1), 122-126.

[26] Lan, Y. T.; Chang, S. C.; Li, A. F.; Lin, T. C.; Chen, W. S.; Jiang, J. K.; Yang, S. H.; Wang, H. S.; Lin, J. K. p53 protein accumulation as a prognostic marker in sporadic colorectal cancer. *Int J Colorectal Dis*. 2007, *22*(5), 499-506.

[27] McGregor, M. J.; Fadhil, W.; Wharton, R.; Yanagisawa, Y.; Presz, M.; Pritchard, A.; Womack, C.; Dutton, S.; Kerr, R. S.; Kerr, D. J.; Johnstone, E. C.; Ilyas, M. Aberrant P53 expression lacks prognostic or predictive significance in colorectal cancer: results from the VICTOR trial. *Anticancer Res*. 2015, *35*(3), 1641-1645.

[28] Munro, A. J.; Lain, S.; Lane, D. P. P53 abnormalities and outcomes in colorectal cancer: a systematic review. *Br J Cancer*. 2005, *92*(3), 434-444.

[29] Ning, S.; Wei, W.; Li, J.; Hou, B.; Zhong, J.; Xie, Y.; Liu, H.; Mo, X.; Chen, J.; Zhang, L. Clinical significance and diagnostic capacity of serum TK1, CEA, CA 19-9 and CA 72-4 levels in gastric and colorectal cancer patients. *J Cancer*. 2018, *9*(3), 494-501.

[30] Zhang, S.-Y.; Lin, M.; Zhang, H.-B. Diagnostic value of carcinoembryonic antigen and

_____

carcinoma antigen 19-9 for colorectal carcinoma. *International journal of clinical and experimental pathology*. 2015, *8*(8), 9404-9409.

[31] Hundt, S.; Haug, U.; Brenner, H. Blood markers for early detection of colorectal cancer: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 2007, *16*(10), 1935-1953.

[32] Lech, G.; Słotwiński, R.; Słodkowski, M.; Krasnodębski, I. W. Colorectal cancer tumour markers and biomarkers: Recent therapeutic advances. *World J Gastroenterol*. 2016, *22*(5), 1745-1755.

[33] Kopylov, A. T.; Stepanov, A. A.; Malsagova, K. A.; Soni, D.; Kushlinsky, N. E.; Enikeev, D. V.; Potoldykova, N. V.; Lisitsa, A. V.; Kaysheva, A. L. Revelation of Proteomic Indicators for Colorectal Cancer in Initial Stages of Development. *Molecules*. 2020, *25*(3).