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Original Article

Does cyclin E and p57^{kip2} expression have prognostic and survival value in colorectal adenocarcinoma?

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Abstract: Introduction: Colorectal cancer is still one of the main causes of cancer death in the world. There is a continous need for novel biomarkers for diagnose, treatment modalities and follow-up. Cyclin E and p57^{KIP2} as the positive and negative regulators of cell cycle seem to be an important target for investigations. Materials and methods: In a retrospective setting, primary colorectal adenocarcinoma cases examined in Mustafa Kemal University, School of Medicine, Pathology Department between 2008-2015 were reviewed. Immunohistochemical expressions of cyclin E and p57^{KIP2} in 80 pairs of colorectal carcinoma and adjacent normal mucosal tissues were evaluated and the findings were compared with clinicopathological parameters and survival time. Results: There were no statistically significant difference between two groups both in cyclin E and p57^{KIP2} stained tissues (P>0.05). There were 40 (50%) patients in high-expression group and 40 (50%) patients in low-expression group for cyclin E. P57^{KIP2} was negative in 55 (68.75%) patients and positive in 25 (31.75%) patients. There were no statistically significant relation between p57^{KIP2} and cyclin E expressions with clinicopathologic parameters defined as age, gender, lymphovascular invasion, perineural invasion, depth of invasion, nodal involvement, emergency in operation, perforation before operation and overall survival except that there was significant relation between p57^{KIP2} expression and histological grade (P=0.012). Conclusions: Immunohistochemical studies of cyclin E and p57^{KIP2} should be performed with larger series of patients supported by more detailed technical research methods to be candidates as predictive markers for treatment modalities and prognostic factors.

Keywords: Cyclin E, p57^{KIP2}, colorectal adenocarcinoma, immunohistochemistry

Introduction

Colorectal cancer is one of the leading causes of cancer death in the world, and according to World Health Organization GLOBOCAN database, incidence and mortality rates of colorectal cancer are 9.7% and 8.5%, respectively (http://globocan.iarc.fr/) [1]. The basic risk assesment and therapeutic approach is accomplished by pathologic stage and histologic subtype with the aid of different biomarkers in newly diagnosed colorectal cancer [2]. But there is a need for new biomarkers for risk assesment, early diagnose, treatment modalities and predicting response to therapy protocols with this lethal disease.

Proteins involved in the control of the cell cycle like cyclins and cyclin-dependent kinases (CDKs) and their inhibitors are among the novel therapeutic targets and are of great interest for new investigations. Among them CDK4-6 inhibitors are new agents targeting the cell cycle and have shown promising effect in breast cancer and gynecologic tumors [3, 4]. The fundemantal ability of cancer cells is to sustain chronic proliferation by supporting the cell cycle independently [5].

Abnormalities in the cell cycle are a leading factor in tumor progression and there are several regulators for governing the cell cycle. Cyclins activate cyclin-dependent kinase and control the progression of cells through the cell cycle [6, 7]. Cyclin E, which includes full-length (FL) cyclin E, low-molecular weight (LMW) cyclin E and total molecular cyclin E (full-length plus low-molecular weight), is one of the most impor-

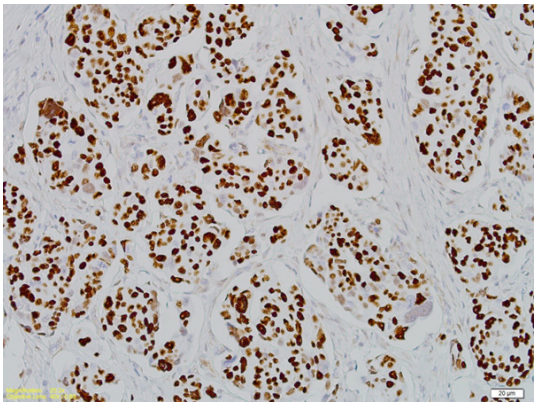

Figure 1. Strong nucleer cyclin E expression in carcinoma tissue (Cyclin E ×200).

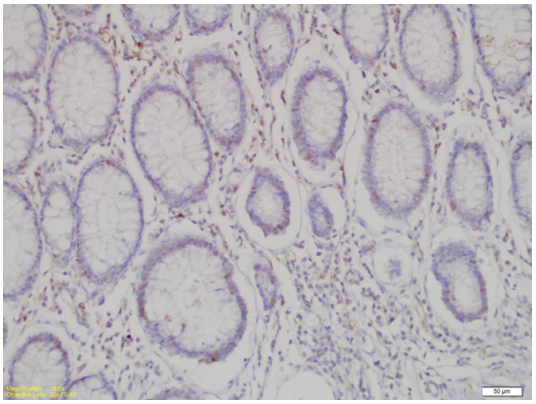

Figure 2. Weak and focal expression of cyclin E in adjacent normal mucosa (Cyclin E ×200).

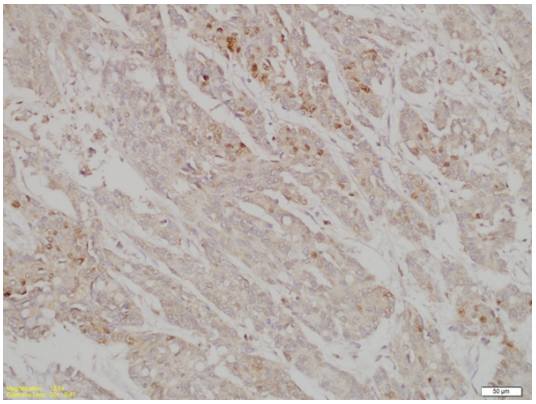

Figure 3. P57^{KIP2} expression of adenocarcinoma (p57^{KIP2} ×200).

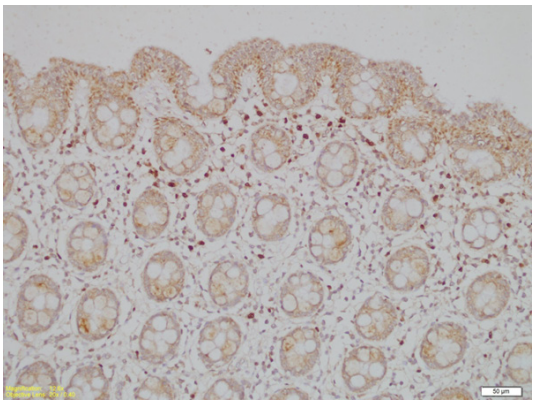

Figure 4. P57^{KIP2} expression in surface epithelium of normal colonic mucosa (p57^{KIP2} ×200).

tant regulators of cell cycle by playing a role in G1 phase, G1-S transition and causing chromosomal instability. Abnormalities in cell cycle may lead to malignant transformation and tumor progression. Notably, cyclin E has been defined as a poor prognostic marker in various solid organ carcinomas including breast. Interestingly overexpression of cyclin E has been related with lower clinical benefit rate and progression-free survival from some recombinant monoclonal antibody therapy agents in advanced breast cancer patients [8, 9]. But there are conflicting data about the role of cyclin E in colorectal adenocarcinoma (CRA) [2, 10, 11].

The gene encoding p57^{KIP2} is on chromosome 11p15. 5 [12], that often undergoes maternal allele loss of heterozygosity in neoplasia. p57^{KIP2} is known to be the negative regulators of cell cycle belonging to KIP family. The CIP/KIP family is composed of p21$^{WAF1/CIP1}$, p27^{KIP1} and p57^{KIP2}. These proteins share a conversed N-terminal domain and inhibit a broad range of CDKs by binding to several cyclin/CDK complexes, including cyclin D/CDK4 (or CDK6), cyclin E/CDK2 and cyclin A/CDK2 contribute to passage of cells through the G1, S, G2, and M phases of the cell cycle [13]. p57^{KIP2} also suppresses cellular transformation by binding to proliferating-cell nuclear antigen [14] inhibits the conversion of conditionally immortal cells to the fully immortal phenotype [15], overexpression of p57^{KIP2} induces cellular senescence, and arrests cells in G, phase completely [12], Its role in carcinogenesis and metastasis to lymph nodes of tumor remains unclear [16].

There are limited data searching the role of p57^{KIP2} in colorectal carcinogenesis with its interaction by another cell cycle regulator; cyclin E [16, 17]. The aim of our study is to search the expression patterns of cell cycle regulators cyclin E and p57^{KIP2} in our CRA seri-

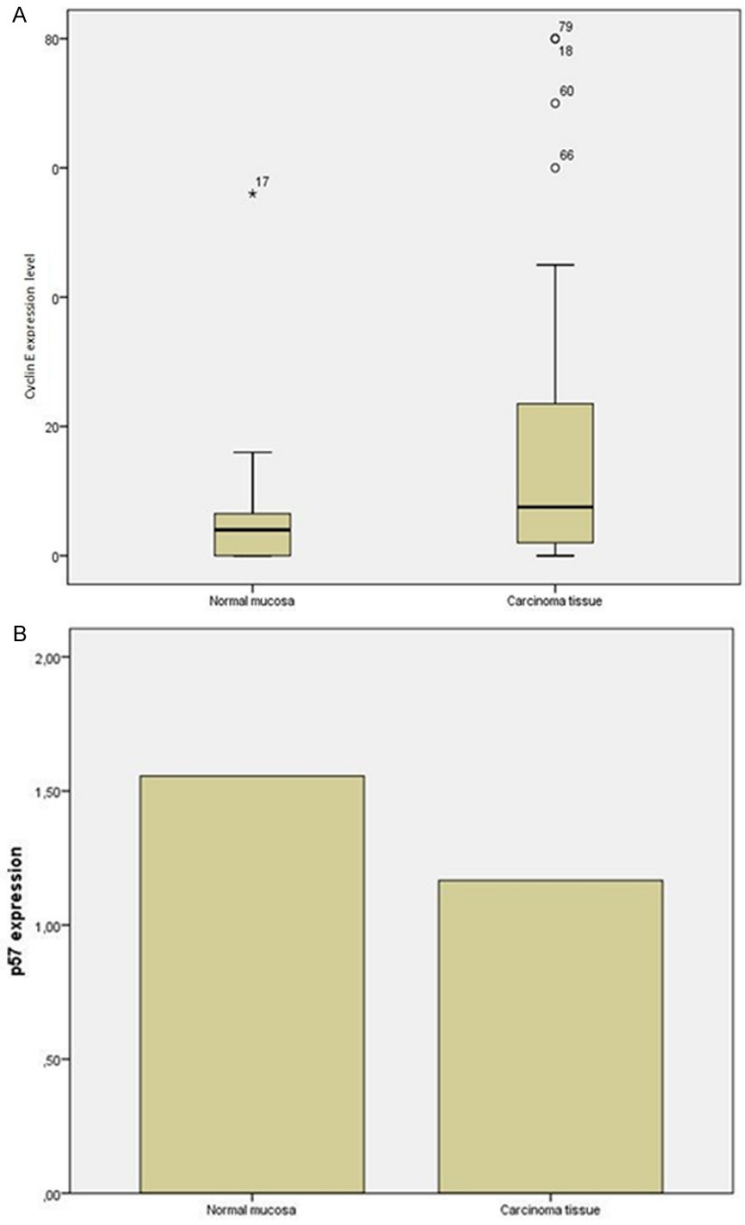

Figure 5. A, B. Cyclin E and p57^{KIP2} expressions in carcinoma and normal mucosa.

es and their relations with clinopathologic parameters and survival rates.

Materials and methods

This was a retrospective archival study including 80 patients diagnosed with colonicadenocarcinoma in the Pathology Department of Mustafa Kemal University, School of Medicine between 2008-2015. Only patients that were diagnosed with resection specimens and had no neo-adjuvant chemotherapy were included in this study.

The ethical committee on human research at our institution approved the protocol for all human research with number 4298783/05001 date 17/01/2015. The haematoxylin eosin (HE) stained cross sections of the cases have been re-evaluated for histopathology on the basis of prognostic factors. The preparate representing the tumor in the best manner was selected.

Sections of 3-4 mm thickness were cut from the paraffin blocks of these preparations and then were de-paraffinized and rehydrated through a graded series of alcohol, microwave antigen retrieval method was used, followed by incubation with cyclin E (HE12): sc-247 (monoclonal mouse antibody, Santa Cruz Biotechnology, CA, USA) and P57^{KIP2} (C-20): sc-1040 (poyclonal rabbit antibody, Santa Cruz Biotechnology, CA, USA).

Immunohistologic staining (IHS) was applied. Cytoplasmic and nuclear staining in normal testis tissue for cyclin E and nuclear staining in fetal kidney for p57^{KIP2} accepted as positive controls. Immunoreactivity scored by nuclear immunostaining in tumor for cyclin E and p57^{KIP2} was calculated as the percentage of positive epithelial cells in relation to the total number of cells encountered in at least 5-10 representative high power fields (500-1000 neoplastic epithelial cells).

The immunoreactivity was interpreted by light microscopy. Staining was evaluated only in areas with well-preserved tissue morphology and away from necrosis or artifact. Staining was found in the nucleus, and tumor cells showed a range of intensities of staining.

Every stained nucleus was considered positive, irrespective of intensity. In agreement with previous studies [11, 18], cases were regarded

Table 1. Association of Cyclin E and p57 expressions with clinicopathologic factors

Variable	Cyclin E expression			p57 expression		
	Low (n)	High (n)	*p* value	Negative (n)	Positive (n)	*p* value
Age	61.37±14.3235	60.20±15.1322	0.722	60.58±14.6675	61.24±14.9088	0.854
Gender						
Male	20	14	0.129	13	12	0.334
Female	20	26		33	22	
Histologic type						
Well, moderate	30	24	0.152	42	12	0.012
Poor, mucinous	10	16		13	13	
Depth of invasion						
T1, T2	4	7	0.339	7	48	0.235
T3, T4	36	33		4	21	
Location						
Colon	31	24	0.091	37	18	0.672
Rectum	9	16		18	7	
Lymph node metastasis						
Absent	20	23	0.453	27	16	0.364
Present	20	17		28	9	
Lymphovascular invasion						
Absent	24	31	0.091	40	15	0.255
Present	16	9		15	10	
Perineural invasion						
Absent	29	31	0.606	44	16	0.126
Present	11	9		119		
Emergency in operation						
Absent	28	32	0.302	40	20	0.486
Present	12	8		15	5	
Perforation at initial evaluation						
Absent	36	35	0.723	50	21	0.365
Present	4	5		5	4	
Liver metastasis						
Absent	35	34	0.745	48	21	0.694
Present	5	6		7	4	

as negative when <2% of the tumour nuclei showed staining or no staining at all (for the detection of cyclin E and p57^{KIP2}).

Statistical analysis

The collected data were analyzed by using the SPSS version 21.0 (SPSS Inc, Chicago, IL, USA). Tumor and non-tumor tissues of colorectal cancer patients were compared by paired t-test and Spearman's correlation test. Clinicopathologic parameters, p57^{KIP2}, and cyclin E comparisons of tumor cases were performed with Pearson Chi-Square non-parametric test. The postoperative survival rate was analyzed by Kaplan-Meier method, and differences in survival rates were assessed with log-rank test. Cox regression test was used for univariate and multivariate analysis. *P* values <0.05 were deemed significant.

Results

Cyclin E and p57^{KIP2} expression were investigated immunohistochemically in CRA patients' cancerous and normal tissues (**Figures 1**-**4**). While the relative expression levels of cyclin E in CRA patients' cancerous and non-cancerous tissues were found to be a mean of 14.9167 and 4.8194, p57^{KIP2} expression levels were 1.1667 and 1.5556, respectively. There were no statistically significant difference between two groups both in cyclin E and p57^{KIP2} stained tissues (P>0.05). But there was apositive correlation between cyclin E and p57^{KIP2} expressions in cancerous tissues (r=0.409) (**Figure 5A**, **5B**).

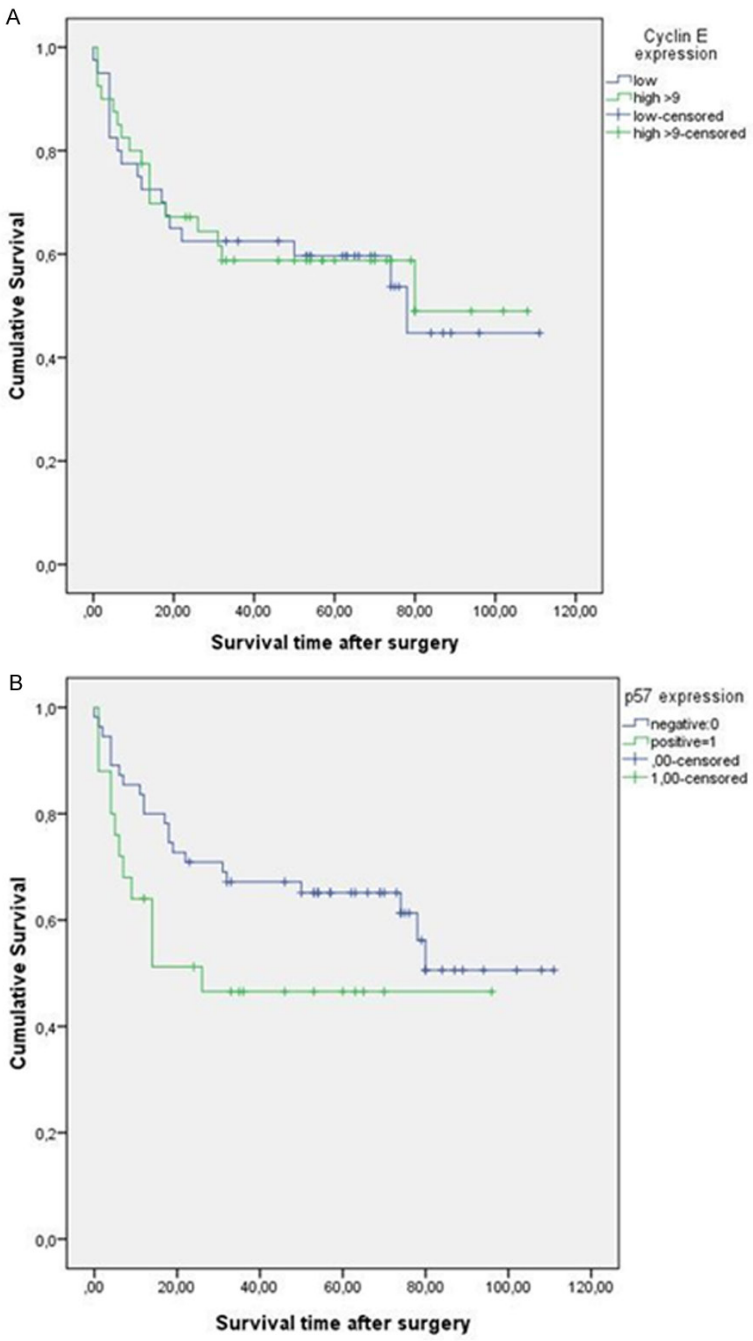

Figure 6. A, B. Kaplan-Meier curves for overall survival in colorectal cancer patients according to Cyclin E and p57^{KIP2} expression.

To study the relationships of clinicopathological characteristics and cyclin E expression with p57^{KIP2} in CRA patients, patients were divided into two groups. Cyclin E expression levels were divided into high and low-expression groups according to mean value, and p57^{KIP2} expression was grouped as negative or positive.

There were 40 (50%) patients in high-expression group and 40 (50%) patients in the low-expression group for cyclin E. The relation between cyclin E expression level and age, gender, lymphovascular invasion, perineural invasion, histological grade, depth of invasion, nodal involvement, emergency in operation, and perforation before operation was investigated and we did not find any statistically significant relation between these parameters and cyclin E relative expression levels. p57^{KIP2} was negative in 55 (68.75%) patients and positive in 25 (31.75%) patients.

There were no statistically significant correrelations between p57^{KIP2} expression and clinicopathologic parameters defined as age, gender, lymphovascular invasion, perineural invasion, depth of invasion, nodal involvement, emergency in operation, and perforation before operation but there was a significant correlation between p57^{KIP2} expression and histological grade (P=0.012) (**Table 1**).

For the relationship of clinicopathological characteristics with cyclin E and p57^{KIP2} expression levels with patients' survival, univariate analysis of clinicopathological factors and cyclin E and p57^{KIP2} was done. Of the 80 patients, 35 (43.48%) died within the follow-up period. The cumulative overall survival rate was 56.3%. There were 7 (43.8%) deaths in the low-cyclin E expression group and 28 (56.9%) in high-expression group. There were 22 (62.9%) deaths in the p57^{KIP2} negative expression group and 12 (37.1%) in the p57^{KIP2} positive expression group (**Figure 6A**, **6B**). Survival time was associated with the depth of invasion (P=0.05, r=-0.313) in the

Table 2. Univariate analysis of clinicopathological factors for overall survival

Variable	Number	Hazard ratio	95% CI	**p value
Age				
≤65	53	1	0.764-3.179	0.223
>65	27	1.558		
Gender				
Male	34	1	0.524-2.790	0.655
Female	46	1.210		
Histologic grade				
Well, moderate	54	1	0.403-2.025	0.804
Poor, mucinous	26	0.903		
Depth of invasion				
T1, T2	11	1	0.359-3.521	0.841
T3, T4	69	1.124		
Location				
Colon	55	1	0.445-3.266	0.714
Rectum	25			
Lymph node metastasis				
Absent	43	1	0.707-2.988	0.309
Present	37	1.454		
Lymphovascular invasion				
Absent	55	1	0.137-3.179	0.603
Present	25	0.659		
Perineural invasion				
Absent	60	1	0.988-19.884	0.052
Present	20	4.443		
Emergency in operation				
Absent	60	1	0.299-2.888	0.898
Present	20	0.929		
Perforation at initial evaluation				
Absent	71	1	0.410-7.776	0.439
Present	9	1.786		
Liver metastasis				
Absent	69	1	0.330-2.931	0.977
Present	11	0.984		

**p<0.05.

p57^{KIP2} group and there was a negative correlation between p57^{KIP2} positive expression group and survival time (r=-0.324). No correlationswere found with other clinicopathologic characteristics both for cyclin E and p57^{KIP2}. (**Table 2**).

Cyclin E and p57^{KIP2} expression levels, clinicopathologic characteristics, and overall survival were searched by Kaplan-Meier analysis, log-rank test, and Cox-regression test. There was no statistically significant correlation (P>0.05) between cyclin E and p57^{KIP2} expression, clinicopathologic characteristics, and overall survival (**Table 2**).

Also, the relation of cyclin E and p57^{KIP2} expressions with carcinomas localised left and right colon was interrogated. There was no statistically significant correlation (P>0.05).

Discussion

There is a need to find new prognostic biomarkers which may be applied in clinical practice for the identification of patients with CRA who have higher survival potential. The detection of these markers may aid in risk stratification and cyclin E with p57^{KIP2} may be included in the list of possible candidates.

Abnormalities in the expressions of cell cycle regulatory genes might lead to neoplastic transformation, dysregulation in positive or negative regulators by aberrant or lost expression, resulting in malignant transformation of cells.

Cyclin E, a positive regulator of the cell cycle, has a leading role in malignant transformation of cancer cells and tumorigenesis due to chromosomal instability with resulting metastasis [19-22].

There are several studies supporting overexpression of cyclin E in CRA carcinogenesis [11, 23-25]. Yasui et al. defined cyclin E expression in 56% of adenocarcinomas; compared to this study we have found higher cyclin E expression rates of 80% in our series [24]. There are some studies demonstrating high expression levels of cyclin E in their CRA patient groups. Some

studies indicate that higher levels of cyclin E expression might be related to gene amplification, chromosomal instability, and play a role in colorectal carcinogenesis [26, 27]. We also have low and high cyclin E expressing CRA groups which might also show the role of these carcinogenesis pathways in our patients.

Bioabnormalities in the mucosa adjacent to tumor are shown in a few reports that included mucosa adjacent to tumor in their studies [22, 25, 28]. Sutter et al. showed overexpression of cyclin E with corresponding normal mucosal samples in their 20 patient series by immunohistochemistry. They defined scattered staining of the nuclei by cyclin E with lower averages compared to tumor tissue [25].

On the other hand Qi et al. explored cyclin E1 expression in 50 rectal cancer and 16 pericarcinoma tissues and found higher protein expression levels in carcinoma compared to pericarcinoma tissues suggesting a synergistic effect of cyclin E in development and progression of rectal cancer [29].

We have also included normal mucosal samples of the same cancer patients and found expression in both of the groups; there was difference in expression levels in tumor and normal mucosal samples but it was not statistically significant. Therefore it is possible to show that there are different alterations in macroscopically normal mucosa of CRA patients. There are conflicting results about cyclin E expression and its correlation with clinicopathological parameters including prognosis. The clinicopathologic characteristics of our patients correlated with low and high expression groups of cyclin E; unfortunately there was not any statistically significant difference. There are also studies that were not successful in showing a correlation [11, 25, 30]. Iochim et al. also detected cyclin E overexpression in 18 of 60 cases but expression did not correlate with conventional clinicopathological features [11].

However, in a study conducted by Li et al., a decrease in cyclin E was associated with some of the parameters like tumor size, mucinous type, venous invasion, and poor prognosis [17]. Moreover, Perea et al. also proved that lack of cyclin E expression might be an indicator of poor prognosis and a marker of advanced stage disease [31]. But it should be cautioned that number in their study group [28] was less than ours (80) which could explain the differences in results among various studies. p57^{KIP2} has an important role in neoplastic transformation of various human cancers but its expression has been shown limited in numbers of studies in CRA [16, 32-34]. Li et al. investigated 189 primary CRA and 22 normal mucosa specimens for p57^{KIP2} immunohistochemically and found a significant decrease in primary carcinomas. Beside this, Noura et al. examined the expression of p57^{KIP2} in 110 pairs of colorectal nontumor and cancer tissues. Their immunohistochemical analysis showed weak expression in normal mucosa compared to cancer tissues [16, 17]. We have also studied cancer and adjacent normal mucosa to compare expression paterns and show the development of neoplasia. We determined p57^{KIP2} in both of the sample groups but unfortunately could not determine a significant difference in expression levels or patern. The limitation of our study was the smaller sample size and a different clone of the immunohistochemical reagent. Some reports have also investigated the relationship of p57^{KIP2} with clinicopathologic parameters and survival time. Li et al. could not show a correlation of p57^{KIP2} with clinicopathologic indices but the patients having loss of this protein tended to show poorer prognosis. Noura et al. found correlation of p57^{KIP2} and large tumor size or female gender. They stated that p57^{KIP2} did not influence the prognosis [16, 17]. We have also searched with the same analysis and determined the relation of p57^{KIP2} with histopathologic differentiation. Interestingly, positive p57^{KIP2} expression was related with poorer survival time.

One of the main goals of our study was to explore the interaction of these two proteins (cyclin E and p57^{KIP2}) in the same CRA cases due to their roles in the cell cycle. The positive correlation between cyclin E and p57^{KIP2} expressions in cancerous tissues (r=0.409) proved the imbalance of positive and negative regulators in the neoplastic process.

To date, studies are investigating right and left colon cancers that are suggested to be oncologically different [35, 36]. We also evaluated cyclin E and p57^{KIP2} expression, grouping our cases according to their localisation as left and right side,searching the role of cancer pathways. There was no statistically significant correlation.

Some aspects of our study did not support our hypothesis. The immunohistochemical methods might be affected by many factors: laboratory conditions, antibody clone, and most importantly number of patients in a single center.

In conclusion; immunohistochemical studies of cyclin E and p57^{KIP2} might have additional roles in colorectal carcinogenesis but before accepting as potential prognostic and predictive biomarkers for CRA, we need larger series of patients supported by more detailed technical research methods.

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Disclosure of conflict of interest

None.

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References

[1] Sumbul AT, Gogebakan B, Ergun S, Batmaci CY, Tonyali O, Yaldiz M. miR-204-5p expression in colorectal cancer: an autophagy-associated gene. Tumor Biol 2014; 35: 12713-719.

[2] Zhou YJ, Xie YT, Gu J, Yan L, Guan GX, Liu X. Overexpression of cyclin E isoforms correlates with poor prognosis in rectal cancer. EJSO 2011; 37: 1078-84.

[3] Corona SP, Ravelli A, Cretella D, Cappelletti MR, Zanotti L, Dester M, Gobbi A, Petronini PG, Generali D. CDK4/6 inhibitors in HER2-positive breast cancer. Crit Rev Oncol Hematol 2017; 112: 208-14.

[4] Kim YT, Zhao M. Aberrant cell cycle regulation in cervical carcinoma.Yonsei Med J 2005; 46: 597-613.

[5] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-74.

[6] Siu KT, Rosner MR, Minella AC. An integrated view of cyclin E function and regulation. Cell Cycle 2012; 11: 57-64.

[7] Koff A, Cross F, Fisher A, Schumacher J, Leguellec K, Philippe M, Roberts JM. Human cyclin E, a new cyclin that interacts with two members of the CDC2 gene family. Cell 1991; 66: 1217-28.

[8] Scaltriti M, Eichhorn PJ, Cortes J, Prudkin L, Aura C, Jiménez J, Chandarlapaty S, Serra V, Prat A, Ibrahim YH, Guzmán M, Gili M, Rodríguez O, Rodríguez S, Pérez J, Green SR, Mai S, Rosen N, Hudis C, Baselga J. Cyclin E amplification/overexpression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients. PNAS 2011; 108: 3761-66.

[9] Keyomarsi K, Tucker SL, Buchholz TA, Callister M, Ding Y, Hortobagyi GN, Bedrosian I, Knickerbocker C, Toyofuku W, Lowe M, Herliczek TW, Bacus SS. Cyclin E and survival in patients with breast cancer. N Engl J Med 2002; 347: 1566-75.

[10] Grabowski P, Schrader J, Wagner J, Hörsch D, Arnold R, Arnold CN, Georgieva I, Stein H, Zeitz M, Daniel PT, Sturm I. Loss of nuclear p27 expression and its prognostic role in relation to cyclin E and p53 mutation in gastroenteropancreatic neuroendocrine tumors. Clin Cancer Res 2008; 14: 7378-84.

[11] Iochim E. Expression patterns of cyclins D1, E and cyclin-dependent kinase inhibitors p21-waf1/cip1, p27kip1 in colorectal carcinoma: correlation with other cell cycle regulators (pRb, p53 and Ki-67 and PCNA) and clinicopathological features. Int J Clin Pract 2008; 62: 1736-43.

[12] Lee MH, Reynisdottir I, Massague J. Cloning of p57KIP2, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. Genes Dev 1995; 9: 639-49.

[13] Diebold J, Dopfer K, Lai M, Lohrs U. Comparison of different monoclonal antibodies for the immunohistochemical assessment of cell proliferation in routine colorectal biopsy specimens. Scand J Gastroenterol 1994; 29: 47-53.

[14] Watanabe H, Pan ZQ, Schreiber-Agus N, DePinho RA, Hurwitz J, Xiong Y. Suppression of cell transformation by the cyclin dependent kinase inhibitor p57KIP2 requires binding to proliferating cell nuclear antigen. Proc Natl Acad Sei U S A 1998; 95: 1392-97.

[15] Nijjar T, Wigington D, Garbe JC, Waha A, Stampfer MR, Yaswen P. p57Kip2 expression and loss of heterozygosity during immortal conversion of cultured human mammary epithelial cells. Cancer Res 1999; 59: 5112-18.

[16] Noura S, Yamamoto H, Sekimoto M, Takemasa I, Miyake Y, Ikenaga M, Matsuura N, Monden M. Expression of second class of KIP protein p57KIP2 in human colorectal carcinoma. Int J Oncol 2001; 19: 39-47.

[17] Li JQ, Wu F, Usuki H, Kubo A, Masaki T, Fujita J, Bandoh S, Saoo K, Takeuchi H, Kuriyama S, Ishida T, Imaida K. Loss of p57KIP2 is associated with colorectal carcinogenesis. Int J Oncol 2003; 23: 1537-43.

[18] Rath-Wolfson L, Bergman M, Ori Y, Goldman A, Ram E, Koren R, Salman H. Expression of cyclin E in stage III colorectal carcinoma. Oncol Lett 2013; 5: 145-48.

[19] Lim YJ, Kim YH, Ahn GH, Chun HK, Jang WY, Lee JH, Son HJ, Rhee PL, Kim JJ, Paik SW, Yoo BC, Rhee JC. Cyclin E, p27 and mutant p53 do not predict the prognosis in AJCC stage II colorectal carcinomas. Korean J Gastroenterol 2004; 44: 314-20.

[20] Bagheri-Yarmand R, Biernacka A, Hunt KK, Keyomarsi K. Low molecular weight cyclin E overexpression shortens mitosis, leading to chromosome missagregation and centrosome amplification. Cancer Res 2010; 70: 5074-84.

[21] Rajagopalan H, Jallepalli PV, Rago C, Velculescu VE, Kinzler KW, Vogelstein B, Lengauer C. Inactivation of hCDC4 can cause chromosomal instability. Nature 2004; 428: 77-81.

[22] Li JQ, Miki H, Ohmori M, Wu F, Funamoto Y. Expression of cyclin E and cyclindependent kinase 2 correlates with metastasis and prognosis in colorectal carcinoma. Hum Pathol 2001; 32: 945-53.

[23] Corin I, Larsson L, Bergström J, Gustavsson B, Derwinger K. A study of the expression of Cyclin E and its isoforms in tumor and adjacent mucosa, correlated to patient outcome in early colon cancer. Acta Oncol 2010; 49: 63-69.

[24] Yasui W, Kuniyasu H, Yokozaki H, Semba S, Shimamoto F, Tahara E. Expression of cyclin E in colorectal adenomas and adenocarcinomas: correlation with expression of Ki-67 antigen and p53 protein. Virchows Arch 1996; 429: 13-19.

[25] Sutter T, Dansranjavin T, Lubinski J, Debniak T, Giannakudis J, Hoang-Vu C, Dralle H. Overexpression of cyclin E protein is closely related to the mutator phenotype of colorectal carcinoma. Int J Colorectal Dis 2002; 17: 374-80.

[26] Kitahara K, Yasui W, Kuniyasu H, Yokozaki H, Akama Y, Yunotani S, Hisatsugu T, Tahara E. Concurrent amplification of cyclin E and CDK2 genes in colorectal carcinomas. Int J Cancer 1995; 62: 25-28.

[27] Donnellan R and Chetty R. Cyclin E in human cancers. FASEB J 1999; 13: 773-80.

[28] Chen LC, Hao CY, Chiu YS, Wong P, Melnick JS, Brotman M, Moretto J, Mendes F, Smith AP, Bennington JL, Moore D, Lee NM. Alteration of gene expression in normal-appearing colon mucosa of APC(min) mice and human cancer patients. Cancer Res 2004; 64: 3694-700.

[29] Qi F, Yuan Y, Zhi X, Huang Q, Chen Y, Zhuang W, Zhang D, Teng B, Kong X, Zhang Y. Synergistic effects of AKAP95, Cyclin D1, Cyclin E1, and Cx43 in the development of rectal cancer. Int J Clin Exp Pathol 2015; 8: 1666-73.

[30] Zhou YJ, Wan FL, Yao LH, Feng LY. Prognostic value of cyclin E and its relation to blood vessel invasion in rectal cancer. Zhonghua Wei Chang Wai Ke Za Zhi 2008; 11: 167-71.

[31] Perea H, Alvaro E, Rodriguez Y, Gravalos C, Sanchez-Tome E, Rivera B, Colina F, Carbonell P, González-Sarmiento R, Hidalgo M, Urioste M. Approach to early onset colorectal cancer: Clinicopathological, familial, molecular and immunohistochemical charecteristics. World J Gastroenterol 2010; 16: 3697-703.

[32] Okamoto A, Hussain SP, Hagiwara K, Spillare EA, Rusin MR, Demetrick DJ, Serrano M, Hannon GJ, Shiseki M, Zariwala M, et al. Mutations in the p16INK4/MTS1/CDKN2, p15INK4B/MTS2, and p18 genes in primary and metastatic lung cancer. Cancer Res 1995; 55: 1448-51.

[33] Bozdogan O, Atasoy P, Batislam E, Basar MM, Basar H. Significance ofp57(Kip2) down-regulation in oncogenesis of bladder carcinoma: an immunohistochemical study. Tumori 2008; 94: 556-62.

[34] Holm R, Forsund M, Nguyen MT, Nesland JM, Trope CG. Expression of p15^{INK4b} and relationship with clinicopathological features and prognosis in patients with vulvar squamous cell carcinoma. PLoS One 2013; 8: e61273.

[35] Lee MS, Menter DG, Kopetz S. Right versus left colon cancer biology: integrating the consensus molecular subtypes. J Natl Compr Canc Netw 2017; 15: 411-19.

[36] Li P, Xiao Z, Braciak TA, Ou Q, Chen G, Oduncu FS. A relationship to survival is seen by combining the factors of mismatch repair status, tumor location and age of onset in colorectal cancer patients. PLoS One 2017; 12: e0172799.