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Relationship between overexpression of ras p21 oncoprotein and *K-ras* codon 12 and 13 mutations in Turkish colorectal cancer patients

Türk kolorektal kanser hastalarında ras p21 aşırı ekspresyonu ve *K-ras* kodon 12 ve 13 mutasyonları arasındaki ilişki

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Background/aims: *The activation of ras family genes plays an important role in colorectal tumorigenesis. We investigated the clinicopathological characteristics and point mutations of K-ras oncogene codons 12/13 and ras p21 expression using paraffin-embedded materials from cancerous and the surrounding normal tissues of 53 colorectal cancer cases.* ***Methods:*** *K-ras codons 12 and 13 point mutations were analyzed by PCR-Single-Strand Conformational Polymorphism (SSCP) and followed by DNA sequencing, while ras p21 expression was evaluated using immunohistochemistry.* ***Results:*** *Mutations of K-ras and overexpression of the ras p21 were detected in 11% and 76% of the tumors, respectively. Ras protein level in tumor was increased an average of 4.6-fold over that of normal mucosa. Ras p21 overexpression did not correlate with any of the clinicopathological parameters examined. K-ras gene mutations were found mostly in the presence of a mucinous component within the tumor (p=0.06). Follow-up data were available for 43 patients. There was no statistically significant correlation between these alterations and patient outcomes.* ***Conclusions:*** *Our data suggest that, apart from K-ras codons 12/13 point mutations, overexpression of the ras family genes is important in the development of the disease but it appears not to be predictive of survival. Furthermore, mucinous secretion in the colorectum may represent a distinct genetic pattern.*

Key words: Colorectal cancer, ras p21 overexpression, K-ras point mutations, survival

Amaç: *Ras gen ailesinin aktivasyonu kolorektal kanser gelişiminde önemli bir rol oynamaktadır. Bu çalışmada, kolorektal kanser ve onların normal çevre dokularının alındığı 53 hastanın parafine gömülü doku blokları kullanılarak ras p21 ekspresyonu ve K-ras onkogeni kodon 12 ve 13 nokta mutasyonları ile tümörün klinikopatolojik karakteristiği araştırılmıştır.* ***Yöntem:*** *K-ras kodon 12 ve 13 nokta mutasyonları PZR-SSCP analizini takip eden DNA dizi analizi; ras p21 ekspresyonu ise immunhistokimya ile değerlendirildi.* ***Sonuçlar:*** *K-ras mutasyonları ve ras p21'in aşırı ekspresyonu sırasıyla %11 ve %76 olarak tespit edildi. Tümördeki ras protein düzeyi normal mukozanın ortalama 4.6 kat üzerinde bulundu. Ras p21 aşırı ekspresyonu herhangi bir klinikopatolojik parametre ile ilişkili değildi. K-ras gen mutasyonları çoğunlukla tümörün müsinöz kompanentinin varlığı ile birlikte görülmüştür. 43 hastanın takip verileri kullanılabilmiştir. Hastaların sağkalımı ve bu değişimler arasında istatistiksel olarak anlamlı bir korelasyon bulunmamıştır.* ***Tartışma:*** *Sonuçlarımız, K-ras kodon 12/13 nokta mutasyonlarının yanı sıra ras gen ailesinin aşırı ekspresyonunun bu hastalığın gelişiminde önemli olduğunu fakat sağkalımın bir ön belirteci olmadığını işaret etmektedir. Ayrıca, kolorektumda müsinöz sekresyon farklı bir genetik patern gösterebilir.*

Anahtar Kelimeler: Kolorektal kanser, ras p21 ekspresyon, K-ras nokta mutasyonları, sağkalım

INTRODUCTION

The *ras* family of genes (H-, K-, and N-*ras*) encodes a 21-kDa membrane protein ($p21^{ras}$), which possesses GTPase activity and participates in a signal transmission pathway (1). A major mechanism of ras-induced oncogenic transformation is related to an enhanced mitogen-activated protein kinase (MAPK) pathway signaling caused by ras-dependent activation of Raf serine/threonine-specific kinases. Oncogenic point mutations in the three human *ras* genes have been detected in a wide variety of human cancers, with carcinomas of the pancreas, colon, thyroid and lung showing the highest incidence (2). Hot spots for *ras* mutations are found in codons 12, 13 and 61, causing the mu-

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tant protein to lose its activity to exchange GTP with GDP, which is related to the promotion of colorectal cancer. Overexpression of the *ras* genes has also been shown to cause a step towards malignant transformation (3).

Previous studies have reported that mutations of the K-*ras* gene are much more likely to occur in the colorectal cancers than other mutations in the ras genes (4). Frequency of K-*ras* mutations and overexpression of the protein in colorectal cancer varies between 14-50% and 29-76%, respectively (5). The most frequently observed mutations on the K-*ras* gene are codons 12 and 13 in the first exon. In several studies, these alterations have been considered as a marker of poor prognosis in patients with colorectal carcinoma (6-8). These findings, however, have not been confirmed to be consistent in several other studies (9-10).

Recently, some studies have shown that the presence of K-*ras* mutations is an important predictive factor for colorectal cancer management. Lievre et al. (11) found that K-*ras* mutation was significantly associated with response to cetuximab therapy. It was also shown that serum K-*ras* mutations are associated with Dukes' stage D and cancer-related survival (12). Moreover, fecal reverse transcription-polymerase chain reaction (RT-PCR) based K-*ras* mutation analysis has been found as a useful technique for the diagnosis of colorectal cancer (13).

In the present study, we analyzed 53 colorectal cancer patients to evaluate the expression of ras p21 oncoprotein and mutations of K-*ras* codons 12/13 and their relationship to clinicopathologic findings. Forty-three of all the tumors were additionally assessed for their prognostic value.

MATERIALS AND METHODS

Patient Specimens

The study protocol was approved by the local ethics committee. Tissue specimens from 53 sporadic colorectal cancers were obtained with consent from previously untreated patients who had curative surgical resection in the Department of General Surgery in Marmara University Hospital between 1990 and 2002. None of the patients received any postoperative adjuvant therapy. The patient group included 31 women and 22 men with ages ranging from 33 to 86 years (median, 64 years). The median follow-up time for 43 patients was 76 months (range, 6-168).

Archival materials were reviewed for each case by an experienced pathologist. For each of the cases in the study, tissue blocks of a representative tumor and a surrounding normal mucosa were selected. All the tumors were defined by stage I-II according to the Tumor Node Metastasis (TNM) system of the International Union Against Cancer (UICC). All the tumors were adenocarcinomas, and the distribution of cases using the World Health Organization (WHO) grading system was as follows: 11 low-, 31 moderate-, and 11 high-grade adenocarcinomas. The tumor was defined as mucinous-component positive when the mucin was presented in more than 20% of the tumor.

DNA Extraction

DNA analysis and immunohistochemistry (IHC) were performed on consecutive sections of the same selected samples, 10% formalin-fixed paraffin-embedded tissue blocks. Genomic DNA was extracted from stored tissues using a method described previously (14). Briefly, the 6 µm-thick sections were cut from blocks that had been selected for maximal tumor content. These sections were deparaffinized by washing with xylene followed with absolute ethanol. The deparaffinized tissues were incubated at 55°C in 150 µL of digestion buffer (0.5 mg/ml proteinase K; 0.05 M Tris-HCl, pH 8.5; 1 mM EDTA; 0.5% Tween 20) for three hours. Then the enzyme was inactivated by heating for 10 minutes at 94°C, and the samples were centrifuged at 12,000 g for 10 minutes and stored at -20°C.

Screening for K-*ras* Mutations

PCR-SSCP (Single-Strand Conformational Polymorphism) is the most often used technique to detect DNA mutations at multiple places in DNA fragments (15). This method contains two steps: making specific PCR and then running the products on the acrylamide gel. Firstly, the region containing codons 12 and 13 of the K-*ras* gene was amplified using oligonucleotide primers with the DNA sequence: 5'-CCCTCCCCCTGATGACTTA-3' (forward) and 5'-GACTGAATATAAACTTGTGG-3' (reverse). After 35 cycles of PCR (94°C for 1 min, 55°C for 1 min and 72°C for 1 min) from 200 ng of sample DNA in 50 µL final volume containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit of *Taq* DNA polymerase and 20 pmole of each primer, the PCR product was analyzed by 1.2% agarose gel electrophoresis.

Secondly, single-stranded DNA for SSCP analysis was produced by combining 3 µL PCR product and 18 µL formamide loading buffer (95% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) and heating at 95˚C for 10 minutes. Silver staining was conducted by use of a modification of the method described previously (16).

Samples showing aberrant migration by screening for K-*ras* mutations were subjected to automated DNA sequencing. Forward primer used for sequencing of K-*ras* gene was the same as those utilized in the PCR-SSCP technique. The PCR products were purified (High Pure PCR Product Purification Kit, Roche) and sequenced on ABI Prism™ 310 fluorescent sequencing analyzer (DYEnamic™ ET Terminator Cycle Sequencing Kit, Amersham Biosciences) and then evaluated with sequencing analysis software.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 4 µm sections of paraffin-embedded tissue blocks that had been mounted on 5-amino-propyltriethoxysilane (AAS)-coated slides and were allowed to dry overnight. After de-waxing in xylene, endogenous peroxidase was blocked with 3% H_2O_2 for 20 minutes. Following antigen retrieval by citrate buffer (pH 6.0) in microwave for 15 minutes, the sections were washed in phosphate-buffered saline (PBS). For blocking nonspecific binding of secondary antibody, Ultra V Block (Ultra Vision Kit; TP-015-HL; Lab Vision) was applied to the sections. Avidin-biotin-peroxidase technique was performed using Ras Ab-1, which reacts with H-, K-, N-*ras* proteins (Cat.# RB-1627-R7, Lab Vision) as primary, and diaminobenzidine (DAB) as the chromogen, according to manufacturers' instructions, was used running in parallel with the known positive and negative controls. IHC staining for ras antibody was scored according to both the intensity and quantity (% positive cells) of staining, visualized at X40 power, based on the previously established criteria (17). The staining was subjectively standardized to be read as follows: 3(+) when the majority of cells (> two-thirds) were stained intensely, 2(+) when between two-thirds and one-third of cells were stained moderately and strongly, and 1(+) when the staining was focal (< one-third) and mild. Scores 2(+) and 3(+) were considered as overexpression of ras protein.

Statistical Analysis

Cancer-specific survival curves were constructed using the Kaplan-Meier method, and the differences between curves were evaluated using the log rank test. The prognostic factors used in the survival analysis were as follows: the age of the patients (≥55 vs. <55), gender, mucin secretion (absent vs. present), grade (poor and moderate vs. well-differentiated), tumor localization (colon vs. rectum), type of invasion (absent vs. present: lymphatic, vascular, perineural invasion), ras p21 protein overexpression (negative vs. positive), and K-*ras* mutation (absent vs. present). The X^2 test was used to test for differences in distribution between groups. *P* values of less than 0.05 were considered statistically significant.

RESULTS

K-*ras* mutations at codons 12 and 13 were observed in six (11%) of the colorectal cancer patients. Five mutations occurred at codon 12; GGT (Gly) to GAT (Asp) transition was identified in two cases and GGT (Gly) to GTT (Val) transition was identified in three cases. GGC (Gly) to GAC (Asp) change was the only transition that was found at codon 13. Only one patient's DNA showed aberrant migration by SSCP analysis, but sequencing analysis showed that this DNA was wild-type. Figure 1 provides an example of mutations detected using SSCP and sequencing.

Immunohistochemical overexpression of ras p21 oncoprotein was detected in 40/53 (76%) samples tested with Ras Ab-1 polyclonal antibody. Four of the six patients with K-*ras* codons 12/13 mutations showed overexpression of ras p21. None of the surrounding normal tissue of tumors showed overexpression. Figure 2 gives an example of ras p21 expression level by Ras Ab-1. Expression level of ras oncoprotein and mutational frequency of the K-*ras* gene and their relationships to the clinicopathological

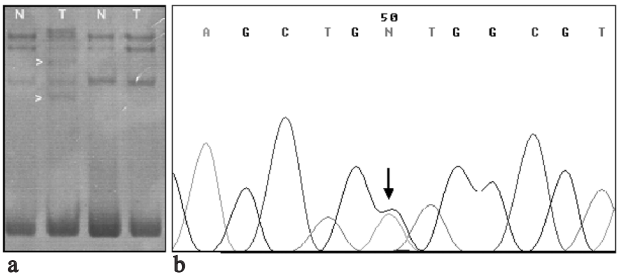

Figure 1. Mutational analysis of K-*ras* gene: **a)** Examples of PCR-SSCP mutation screening for exon 1 of K-*ras* gene. Mutations are indicated by arrows. N: Normal tissue. T: Tumor tissue. **b)** Sequencing of mutant K-*ras* identified by SSCP screening. A wild-type codon 12 (GGT) changed to mutant codon (GAT). Mutation is shown by arrow.

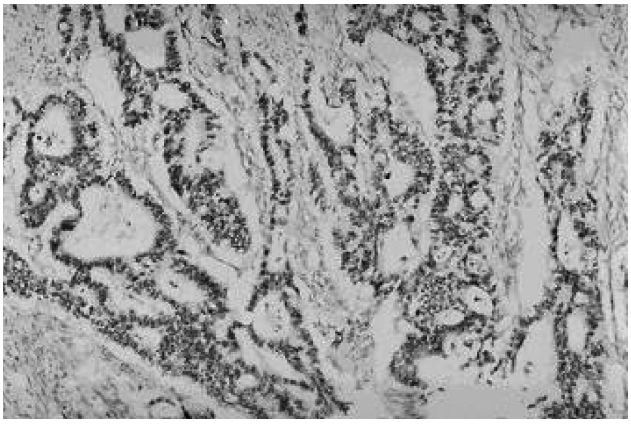

Figure 2. Well-differentiated colorectal adenocarcinoma, with 3(+) ras p21 overexpression (original magnification X20).

findings of the patients are shown in Table 1. Additionally, we found that ras p21 overexpression level in tumor was detected as an average of 4.6-fold over that of normal mucosa.

Of the 43 patients with 6 to 168 months follow-up, eight died of their tumor and 14 recurrences were observed. The overall survival (OS) and disease-free survival (DFS) rates of K-*ras* mutations and ras p21 overexpression are shown in Table 2. There was no statistically significant relationship between these changes and the clinical outcome.

DISCUSSION

The *ras* gene family consists of three homologous genes, *K*-, *H*-, and *N*-*ras*, which encode similar 21-kD protein ($p21^{ras}$) involved in G protein-mediated signal transduction. Aberrations in *ras* genes lead to increased and uncontrolled cell proliferation and malignant transformation. The most exhaustedly worked *ras* gene in colorectal cancer is K-*ras*. We did not find any correlation between the expression level of ras oncoprotein and K-*ras* mutations and patients' clinicopathological parameters, such as age, sex, stage, differentiation, type of invasion, and survival status; however, K-*ras* mutated colorectal tumors did not show a higher mucin production (p=0.06).

In this study, oncogenic mutation at first exon of K-*ras* gene was observed in 11% of TNM stage I-II tumors studied. This value is lower than in most studies of colorectal carcinoma (25-40%) (2). The risk for selection of normal material for PCR

Table 1. Relationship between ras alterations and clinicopathologic findings

Parameters	No. of patients n (%)	ras p21 overexpression n (%)	*p value*	K-*ras* mutation n (%)	*p value*
All patients	53 (100)	40 (76)		6 (11)	
Sex					
Female	31 (58)	25 (81)	*0.30*	4 (13)	*1.0*
Male	22 (42)	15 (68)		2 (9)	
Age (years)					
<55	14 (26)	12 (86)	*0.48*	0 (0)	*0.18*
≥55	39 (74)	28 (72)		6 (15)	
Stage (TNM)					
Stage I	17 (32)	11 (65)	*0.3*	2 (12)	*1.0*
Stage II	36 (68)	29 (80)		4 (11)	
Differentiation					
Good	11 (21)	7 (64)		1 (9)	
Moderate	31 (58)	25 (81)	*0.51*	4 (13)	*0.91*
Poor	11 (21)	8 (73)		1 (9)	
Tumor Localization					
Colon	37 (70)	30 (81)	*0.18*	5 (14)	*0.65*
Rectum	16 (30)	10 (62)		1 (6)	
Mucinous component					
Yes	16 (30)	13 (81)	*0.51*	4 (25)	*0.06*
No	37 (70)	27 (72)		2 (5)	
Type of Invasion					
- Lymphatic					
Yes	16 (30)	14 (88)	*0.3*	1 (6)	*0.7*
No	37 (70)	26 (70)		5 (14)	
- Vascular					
Yes	8 (15)	7 (88)	*0.7*	0 (0)	*0.6*
No	45 (85)	33 (73)		6 (13)	
- Perineural					
Yes	4 (8)	2 (50)	*0.3*	0 (0)	*1.0*
No	49 (92)	38 (78)		6 (12)	

Table 2. Prognostic value of the changes (n=43)

Variable	No of cases	Disease-free survival		P**	Overall survival		P**
		No of recurrence (%)	Mean survival, Mo (95% CI)*		No of deaths (%)	Mean survival, Mo (95% CI) *	
K-*ras*							
Mutant	5	2 (40)	41 (20 to 62)	0.22	1 (20)	117 (68 to 166)	0.63
Normal	38	12 (32)	119 (97 to 142)		7 (18)	140 (121 to 159)	
Ras p21							
Overexpression	33	9 (27)	123 (100 to 148)	0.3	6 (18)	138 (117 to 159)	0.9
Normal	10	5 (50)	70 (45 to 96)		2 (20)	120 (90 to 150)	

*CI: Confidence interval. **Log rank test. P values.

analyses was minimized in our study by careful microscopic examination. The lower values are most probably due to the fact that mutations at other sites of the K-*ras* gene, such as most frequently observed codons 59, 61, 63, might also have been affected. On the other hand, our results supported the significance of recently identified novel K-*ras* mutations. In accordance with our results, K-*ras* A146 mutations were detected in patients and also cell line samples (18). Another identified and characterized novel K-*ras* mutation is G to T transversion at the third position of codon 19 (19). These results may also be explained by the ethnic variations, and differences in the dietary components and lifestyle factors. K-*ras* mutation spectrum might also have been affected by these factors (20, 21).

The level of ras p21 expression that we determined was similar to levels reported in previous studies (22, 23). The main activating mechanism of *ras* genes seems to be the aberrant expression of all three members of *ras* family genes. Abnormal expression of *ras* genes may be attributed either to mutations, not in the coding regions, but within the promoters of these genes (24), or to imbalance of chromosomes carrying these genes, resulting in the gene amplification (25). In our study, there was no significant correlation between ras p21 overexpression and K-*ras* mutations. This may be explained by the fact that Ras Ab-1 monoclonal antibody used in our IHC analysis detects all overexpressed forms of the protein, including the mutant or wild-type.

Our results indicated that the frequency of K-*ras* mutation was higher in colorectal cancer with mucinous component than that of non-mucinous carcinomas (p=0.06). One of the three mucinous carcinomas (33%) had K-*ras* mutation. Thus, it appears that K-*ras* mutations may influence the presence of mucinous component in colorectal carcinomas (26, 27).

Our findings revealed that none of the studied histopathological features was related to ras p21 overexpression. The observations of an increased level of ras p21 expression and its association with none of the known prognostic factors in colorectal tumors suggest that ras overexpression may be important in colorectal carcinogenesis, but not in its progression in stage I and II colorectal carcinomas.

Prognostic analysis for K-*ras* mutational activation and ras p21 immunostaining showed that none of these parameters was predictive of survival. Some studies have indicated the importance of *ras* alterations in predicting long-term outcome, while others have failed to show such a relationship. Collaborative RASCAL studies reported a correlation between specific K-*ras* mutations and poor prognosis (7, 8). Since we only had a limited number of K-*ras* mutant patients, we did not analyze this correlation. The ras overexpression has been associated with poor prognosis in colorectal cancer in some studies (23, 28), and with favorable prognosis in one study (22). However, the mutational status of the K-*ras* gene was not clarified in any of these studies.

Our recently published study showed that neither K-*ras* nor p53 and *DCC* gene alterations play a critical role in patient outcome in the Turkish colorectal cancer population (29).

A large population-based study showed that K-*ras* mutations were more likely to occur in advanced stage and distant and/or regional metastasis. That study also found that there is no association between K-*ras* mutations and disease-free survival (30). ASCO 2006 guidelines do not recommend *ras* oncogene usage for screening, diagnosis, staging, and surveillance, or for monitoring the treatment of colorectal cancer patients due to current insufficient data (31).

In conclusion, there were no significant correlations between ras p21 expression level and first

exon of K-*ras* mutations with the clinicopathological variables. High frequency of K-*ras* mutations in codons 12 and 13 was related to the occurrence of a mucinous component within the tumor suggesting that these mutations may be important in the development of mucinous carcinomas. Our findings failed to demonstrate any statistically significant association between these ras aberrations and patient outcome. Increased level of ras p21 oncoprotein may represent the development of colorectal cancer, but it appears not to be responsible for its progression.

REFERENCES

1. Barbacid M. Ras genes. Ann Rev Biochem 1987; 56: 779-827.
2. Bos JL. Ras oncogenes in human cancers. Cancer Res 1989; 49: 4682-9.
3. Spandidos DA, Wilkie NM. Malignant transformation of early passage rodent cells by a single mutated human oncogene. Nature 1984; 310: 469-75.
4. Bos JL, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. Nature 1987; 327: 293-7.
5. McDermott U, Longley DB, Johnston PG. Molecular and biochemical markers in colorectal cancer. Ann Oncol 2002; 13 (Suppl 4): 235-45.
6. Conlin A, Smith G, Carey FA, et al. The prognostic significance of K-*ras*, p53, and APC mutations in colorectal carcinoma. Gut 2005; 54: 1283-6.
7. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten *ras* mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst 1998; 90: 675-84.
8. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten *ras* mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer 2001; 85: 692-6.
9. Bouzourene H, Gervaz P, Cerottini JP, et al. p53 and K-*ras* as prognostic factors for Dukes' stage B colorectal cancer. Eur J Cancer 2000; 36: 1008-15.
10. Petrowsky H, Sturm I, Graubitz O, et al. Relevance of Ki-67 antigen expression and K-*ras* mutation in colorectal liver metastases. Eur J Surg Oncol 2001; 27: 80-7.
11. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006; 66: 3992-5.
12. Trevisiol C, Di Fabio F, Nascimbeni R, et al. Prognostic value of circulating KRAS2 gene mutations in colorectal cancer with distant metastases. Int J Biol Markers 2006; 21: 223-8.
13. Chien CC, Chen SH, Liu CC, et al. Correlation of K-*ras* codon 12 mutations in human feces and ages of patients with colorectal cancer (CRC). Transl Res 2007; 149: 96-102.
14. Soong R, Iacopetta BJ. A rapid and nonisotopic method for the screening and sequencing of p53 gene mutations in formalin-fixed, paraffin-embedded tumors. Mod Pathol 1997; 10: 252-8.
15. Orita M, Iwahana H, Kanazawa H, et al. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci U S A. 1989; 86: 2766-70.
16. Bassam BJ, Caetano-Anolles G, Gresshoff PM. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 1991; 196: 80-3.
17. Lee CS. Ras p21 protein immunoreactivity and its relationship to p53 expression and prognosis in gallbladder and extrahepatic biliary carcinoma. Eur J Surg Oncol 1997; 23: 233-7.
18. Edkins S, O'Meara S, Parker A, et al. Recurrent KRAS codon 146 mutations in human colorectal cancer. Cancer Biol Ther 2006; 5: 928-32.
19. Akagi K, Uchibori R, Yamaguchi K, et al. Characterization of a novel oncogenic K-*ras* mutation in colon cancer. Biochem Biophys Res Commun 2007; 352: 728-32.
20. Slattery ML, Anderson K, Curtin K, et al. Lifestyle factors and Ki-ras mutations in colon cancer tumors. Mutat Res 2001; 483: 73-81.
21. Wark PA, Van der Kuil W, Ploemacher J, et al. Diet, lifestyle and risk of K-*ras* mutation-positive and -negative colorectal adenomas. Int J Cancer 2006; 119: 398-405.
22. Hirvikoski P, Auvinen A, Servomaa K, et al. K-*ras* and p53 mutations and overexpressions as prognostic factors in female rectal carcinoma. Anticancer Res 1999; 19: 685-92.
23. Sun XF, Wingren S, Carstensen JM, et al. ras p21 expression in relation to DNA ploidy, S-phase fraction and prognosis in colorectal adenocarcinoma. Eur J Cancer 1991; 27: 1646-9.
24. Chakraborty AK, Cichutek K, Duesberg PH. Transforming function of proto-ras genes depends on heterologous promoters and is enhanced by specific point mutations. Proc Natl Acad Sci USA 1991; 88: 2217-21.
25. Duesberg PH. Oncogenes and cancer. Science 1995; 267: 1407-8.
26. Zhang H, Evertsson S, Sun X. Clinicopathological and genetic characteristics of mucinous carcinomas in the colorectum. Int J Oncol 1999; 14: 1057-61.
27. Lin JK, Chang SC, Wang HS, et al. Distinctive clinicopathological features of Ki-ras mutated colorectal cancers. J Surg Oncol 2006; 94: 234-41.
28. Miyahara M, Saito T, Kaketani K, et al. Clinical significance of ras p21 overexpression for patients with an advanced colorectal cancer. Dis Colon Rectum 1991; 34: 1097-102.
29. Akkiprik M, Ataizi-Çelikel Ç, Düşünceli F, et al. Clinical significance of p53, K-*ras* and DCC gene alterations in the stage I-II colorectal cancers. J Gastrointestinal Liv Dis 2007; 16: 11-7.
30. Samowitz WS, Curtin K, Schaffer D, et al. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev 2000; 9: 1193-7.
31. Locker GY, Hamilton S, Harris J, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol 2006; 24: 5313-27.