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Molecular Detection of *APC*, K-*ras,* and *p53* Mutations in the Serum of Colorectal Cancer Patients as Circulating Biomarkers

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Abstract. Early detection of tumor DNA in serum/plasma prior to the development of recurrence or metastases could help improve the outcome of patients with colorectal cancer (CRC) after tumor resection. Recent advances in the detection of tumor DNA in the serum/plasma has opened up numerous new areas for investigation and new possibilities for molecular diagnosis. *APC* and K-*ras* mutations are considered to be early-stage developments of CRCs, whereas *p53* mutations are thought to be relatively late events in the tumorigenesis of CRCs. The aim of this study was to search for the presence of genetic mutations in the DNA extracted from the serum of CRC patients and healthy subjects. We simultaneously evaluate the significance of *APC*, K-*ras,* and *p53* gene mutations in cancer tissues and their paired serum samples of 104 CRC patients by polymerase chain reaction-single strand conformation polymorphism analysis (PCR-SSCP) followed by direct sequencing. Additionally, analysis was carried out to detect the serum carcinoembryonic antigen (CEA) levels in CRC patients. Overall, we found at least one of the gene mutations in tumor tissues from 75% (78/104) of the CRC patients. Comparison of the three molecular markers showed that the detection rates in the serum were 30.4%, 34.0%, and 34.2% for *APC*, K-*ras,* and *p53* genes, respectively. Of these patients, 46.2% (36/78) were identified as having positive serum results, whereas all healthy controls remained negative. The overall positive tumor DNA detection rates in the serum were 0% (0/7) for Dukes' A classification, 22.4% (11/49) for Dukes' B, 48.7% (19/39) for Dukes' C, and 66.7% (6/9) for Dukes' D. The detection rate increased as the tumor stage progressed ($p = 0.012$). Concurrently, a significant difference was observed between lymph node metastases and positive serum tumor DNA detection ($p < 0.001$). A significantly higher postoperative metastasis/recurrence rate in patients harboring gene mutations with serum tumor DNA than those without serum tumor DNA was also demonstrated ($p < 0.001$). However, no significant correlation between the postoperative metastasis/recurrence and serum CEA levels was observed ($p = 0.247$). These data suggest that the identification of circulating tumor DNA using the molecular detection of *APC*, K-*ras,* and *p53* gene mutations is a potential tool for early detection of postoperative recurrence/metastases. Moreover, these genes may be potential molecular markers of poor clinical outcome in CRC patients.

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Colorectal cancer (CRC) is one of the leading malignancies worldwide and is the second most frequent cause of cancer death in developed countries [1, 2]. Even with the recent advances in diagnostic and surgical techniques, the outcome remains poor in patients with advanced disease, and only CRCs diagnosed at an early stage are likely to be cured by surgical resection [3, 4]. Because distant metastases are the major problems when treating CRC, the development of a sensitive, specific, convenient diagnostic method for detecting CRC at an early stage could ultimately affect the patient's prognosis.

The most common routes of metastases for CRC are usually lymphatic spread to regional lymph nodes and hematogenous spread to the liver through the portal vein. Hematogenous dissemination is presumably a major route of distant metastases in various malignancies, and circulating tumor cells may remain dormant for a long time before clinical metastasis or recurrence [5–7]. Previous reports have indicated that tumors can release DNA into the circulation [8, 9]. According to these studies, it is possible to detect tumor-specific DNA in the serum and plasma of patients with various cancers [10–12]. Hence, detection of such circulating tumor DNA is of utmost importance for the identification and development of reliable diagnostic and prognostic markers of early-stage CRC. Additionally, this is likely to provide clinicians with a crucial predictive tool with respect to recurrence and metastases and to result in a more appropriate selection of patients for adjuvant therapy.

A series of genetic alterations have been associated with CRC, and a multistep model of tumorigenesis involving activation of proto-oncogenes and inactivation of tumor suppressor genes has been proposed [13, 14]. Of the genes characterized to date, inactivation of tumor suppressor genes *APC* and *p53*, as well as activation of the oncogene K-*ras*, are thought to be particularly important determinants of tumor initiation and progression [14]. More recently, it has been shown that replication errors caused by germline or somatic mutations of mismatch repair genes may contribute to the

neoplastic process [15, 16]. To date, although genetic changes associated with colorectal carcinogenesis have been well characterized, little is known about its association with circulating tumor DNA in the serum of CRC patients as a diagnostic application. Therefore in the present study we determined the presence of *APC*, K-*ras,* and *p53* gene mutations in the same series of patients and performed a paired comparison between tumor tissues and serum in individual patients as a potential approach to a noninvasive method for detecting postoperative recurrence/metastasis.

Materials and Methods

Sample Collection and DNA Extraction

The CRC tissues and their corresponding normal tissues were collected from 104 nonselected patients who underwent surgical resection for CRC between May 2001 and May 2002 at the Department of Surgery, Kaohsiung Medical University Hospital. All tissue samples were collected immediately after surgical resection, frozen instantly in liquid nitrogen, and then stored in a −80°C freezer until analyzed. Fifty normal healthy subjects were studied as negative controls.

Genomic DNA from all 104 CRCs and the paired normal tissues were isolated using proteinase-K (Stratagene, La Jolla, CA, USA) digestion and a phenol/chloroform extraction procedure according to the method proposed by Sambrook et al. [17]. Additionally, 5 ml samples of peripheral blood were obtained from the 104 CRC patients at the time of surgical resection and were also obtained from the 50 healthy volunteers. Serum was separated and stored at −20°C before the DNA extraction.

DNA from serum samples was purified using the Puregene kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Serum samples were screened for mutations in all three genes if any gene mutation was identified in tissue samples.

Written informed consent was obtained from all subjects or their guardians (or both) for the use of their resected specimens and blood samples. The acquisition and subsequent use of samples were approved by the institutional review board.

All the patients were followed up regularly at 3- to 6-month intervals; physical examinations, routine blood work, serum carcinoembryonic antigen (CEA) assay, and liver function tests were conducted as appropriate. Chest radiography and abdominal ultrasonography were performed every 6 months. Computed tomography or magnetic resonance imaging was carried out if indicated. The correlation between the development of clinical metastases/recurrence and the detection of serum tumor DNA was analyzed during a median follow-up of 20 months (mean ± SD, 19.7 ± 6.3 months).

Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) Analysis

Polymerase chain reaction (PCR) amplification with single-strand conformation polymorphism (SSCP) analysis of DNA samples (20 ng) was performed at a final concentration of 1 X PCR buffer [Tris-HCl 10 mmol/l, pH 8.3; $MgCl_2$, 1.5 mmol/l; KCl 50 mmol/l; and 0.01% gelatin] with deoxynucleotide triphosphate 100 mmol/l (Promega, Madison, WI, USA) and 5 units (1 unit/μl) of BIOTOOLS DNA polymerase (Biotechnological & Medical Laboratories, Madrid, Spain) in a total volume of 50 μl.

APC. The exon 15 mutations cluster region (codons 1254–1631) for the *APC* gene was analyzed according to Aust et al. with slight modification [18]. Three segments of the *APC* gene were amplified using the oligonucleotide primers as follows: 5′-TAATACGACTCACTATAGGGGGCTGCCACTTGCAAAGTTTCTTCT-3′ (forward) and 5′-TGGCAATCGAAACGACTCTCAAAACT-3′ (reverse) were used to amplify a 494-bp PCR product of codons 1254–1394; 5′-TAATACGACTCACTATAGGGGAGATGTACTTCTGTCAGTTCACT-3′ (forward) and 5′-GGTGGCAAAATGTAATAAAGTATCA-3′ (reverse) were used to amplify a 346-bp PCR product of codons 1394–1484; and 5′-TAATACGACTCACTATAGGGGCAGCCTAAAGAATCAAATGAAAAC-3′ (forward) and 5′-GTGGCATATCATCCCCCGGTGTAAA-3′ (reverse) were used to amplify a 306-bp PCR product of codons 1549–1631. The PCR conditions were as follows: 35 cycles of 20 seconds of denaturation at 94°C, 1 minute of annealing at 60°C, 30 seconds of extension at 74°C, and 5 minutes of final extension at 72°C.

K-ras. The oligonucleotide primers for exons 1 and 2 of the K-*ras* gene were used according to our previously designed sequences [19].

p53. The oligonucleotide primers were designed from p53 coding regions (exons 4–8) as previously described [20]. Subsequently, PCR products were heated at 94°C for 4 minutes and loaded onto polyacrylamide gels of GeneGel Excel 12.5/24 kit (Pharmacia Biotech, San Francisco, CA, USA) using the Genephore electrophoresis unit (Pharmacia Biotech). Gels were run for 5 to 6 hours at 5 to 6 watts and were then stained with DNA silver staining kit (Pharmacia Biotech).

Direct Sequencing

The PCR products were purified by the QIAEX II Gel Extraction Kit (Qiagen, Valencia, CA, USA) and then subjected to sequencing using a double-stranded cycle sequencing system (GIBCO, Gaithersburg, MD, USA). The purified products were then sequenced directly with a T7 promoter/IRD800 (LI-COR, Lincoln, NE, USA), which is a T7 promoter primer labeled with a heptamethine cyanine dye, or using DNA polymerase incorporating IRD-labeled dATP for the sequencing reaction. Upon completion of the sequencing reaction, 4 μl of formamide loading buffer was added to the reaction mixture; the sample was heated to 95°C for 5 minutes, snap-cooled, and loaded onto the sequencing gel.

An automated DNA electrophoresis system (model 4200; LI-COR) with a laser diode emitting at 785 nm and fluorescence detection between 815 and 835 nm was used to detect and analyze the sequencing ladder. Electrophoresis was performed on a 41 cm × 25 cm × 0.2 mm gel consisting of 6% Long Ranger gel matrix (AT Biochem; Malvern, PA, USA) with 7 M urea and a running buffer consisting of 133 mM Tris base, 44 mM boric acid, and 2.5 mM EDTA, pH 9, at 50°C. From each sequencing reaction, 1.5 μl of the sample was loaded on each lane. Following the loading of samples, electrophoresis was carried out at a constant voltage of 2000 V with the gel heated to 50°C. Data collection and image

Table 1. Clinicopathologic characteristics of 104 colorectal cancer patients.

	Data
Sex	
Male	51 (49%)
Female	53 (51%)
Tumor	
< 5 cm	48 (46.2%)
≥ 5 cm	56 (53.8%)
Dukes' stage	
A	7 (6.7%)
B	49 (47.1%)
C	39 (37.5%)
D	9 (8.7%)
Differentiation	
Well	19 (18.3%)
Moderate	60 (57.7%)
Poor	25 (24%)
Tumor location	
Right	27 (26%)
Left	41 (39.4%)
Rectum	36 (34.6%)

Table 2. Genetic biomarkers for the detection of colorectal carcinomas in the serum.

Genetic alteration	Occurrence in tumor	Occurrence in serum	Positive serum biomarkers
APC	46/104 (44.2%)	14/104 (13.5%)	14/46 (30.4%)
K-*ras*	47/104 (45.2%)	16/104 (15.4%)	16/47 (34.0%)
p53	38/104 (36.5%)	13/104 (12.5%)	13/38 (34.2%)
Overall[a]	78/104 (75.0%)	36/104 (34.6%)	36/78 (46.2%)

[a]Mutation occurred in at least one of three genes.

Table 3. Presence of molecular alterations in serum as a function of tumor stage.

Dukes' stage	*APC*	K-*ras*	*p53*	Overall[a]
A	0/3	0/2	0/2	0/7
B	4/20 (20.0%)	4/19 (21.5%)	3/15 (20.0%)	11/49 (22.4%)
C	9/20 (45.0%)	11/24 (45.8%)	6/14 (42.9%)	19/39 (48.7%)
D	1/3 (33.3%)	1/2 (50.0%)	4/7 (57.1%)	6/9 (66.7%)

[a]Mutation occurred in at least one of three genes.

Table 4. Relation of molecular marker expression with clinicopathologic parameters of colorectal cancer patients harboring gene mutations.

Parameter	Circulating DNA detectable patients (n = 36)	Circulating DNA undetectable patients (n = 42)	p
Age (years)[a]	62.1 ± 2.0	65.9 ± 1.9	0.095
Sex			
Male	18	20	0.834
Female	18	22	
Lymph node metastasis			
Absent	11	27	< 0.001
Present	25	15	
Dukes' stage			
A	0	4	0.012
B	11	23	
C	19	13	
D	6	2	
Tumor differentiation			
Well	5	7	0.902
Moderate	22	26	
Poor	9	9	

[a]Mean ± SE.

analysis were performed using an IBM486 (model 90) with the Base Image IR software that accompanied the model 4200 DNA sequencer.

Detection of Serum CEA

Serum samples from 104 CRC patients were obtained less than 1 week before operation. Serum CEA levels were determined by an enzyme immunoassay test kit (DPC Diagnostic Product, Los Angeles, CA, USA), using a value of 5 ng/ml as positive.

Statistical Analysis

All data were analyzed using the Statistical Package for the Social Sciences Version 10.0 software (SPSS, Chicago, IL, USA). The two-sided Pearson χ^2 test and Student's t-test were used to compare the other clinicopathologic parameters between circulating DNA-detectable (DD) patients and DNA-undetectable (DU) patients. The association between the development of clinical recurrence/metastases and the presence of serum tumor DNA or serum CEA levels was also evaluated by the two-sided Pearson χ^2 test. A probability of less than 0.05 was considered statistically significant.

Results

The clinicopathologic characteristics of 104 CRC patients are summarized in Table 1. The pathologic stage was defined according to the modified Dukes' classification [21]. Of the 104 patients with CRC, 75% (78/104) of the tumors were found to contain at least one gene mutation, and only 4.8% (5/104) contained mutations in the *APC*, K-*ras,* and *p53* genes. Table 2 shows these three genetic biomarkers for detecting circulating tumor DNA from the serum of CRC patients. *APC*, K-*ras,* and *p53* mutations were detected in serum samples in 30.4%, 34.0%, and 34.2% of patients, respectively, with tumors harboring the same mutations. The presence of at least one molecular alteration in the serum was observed in 46.2% (36/78) of the cases. In contrast, no mutated *APC*, K-*ras,* or *p53* DNA was detected in serum samples from the 50 healthy volunteers.

With regard to tumor stage, we observed a tendency toward a stage-dependent difference in the occurrence of *APC*, K-*ras,* and *p53* mutations in the serum samples of CRC patients (Table 3). Overall, the presence of at least one marker alteration was observed in 0% (0/7) in Dukes' A, 22.4% (11/49) in Dukes' B, 48.7% (19/39) in Dukes' C, and 66.7% (6/9) in Dukes' D classes. The association of gene mutations as molecular markers for CRC patients with clinicopathologic parameters is depicted in Table 4. Except for the lymph node metastasis and tumor stage, there were no statistical differences in the other clinicopathologic features between DD patients and DU patients.

As shown in Table 4, 25 of 36 (69.4%) DD patients exhibited lymph node metastasis, whereas only 15 of 42 (35.7%) DU patients had lymph node metastasis. There was a significant difference between the two groups ($p < 0.001$). Advanced stages of CRC were detected more significantly in DD patients than in DU patients ($p = 0.012$).

Table 5. Correlation between the detection of molecular markers in colorectal cancer patients harboring gene mutations and the development of postoperative metastases/recurrence.

	Recurrence/metastases		
Marker	Absent ($n = 47$)	Present ($n = 31$)	p
Serum tumor DNA			
Negative	38	4	< 0.001
Positive	9	27	
Serum CEA levels			
< 5 ng/ml	26	13	0.247
≥ 5 ng/ml	21	18	

CEA: carcinoembryonic antigen.

As shown in Table 5, a significant difference existed between the presence of serum tumor DNA and the occurrence of clinical metastases/recurrence postoperatively. Of the 36 DD patients, 27 developed metastases/recurrence after surgical resection of the tumor, whereas only 4 of the 42 DU patients developed recurrence/metastases ($p < 0.001$). No statistical significance was observed between serum CEA levels and postoperative recurrence/metastases ($p = 0.247$).

Discussion

Recurrence and distant metastasis are still major problems when treating solid tumors. Recurrence following curative resection of apparently localized CRC suggests that undetectable disseminated tumor cells may have been present at the time of operation. In early-stage malignancies, disseminated tumor cells are not yet capable of forming metastases. However, such tumor cells or DNA circulating in peripheral blood may represent an easily sampled source for early cancer detection, thereby improving the selection of a therapeutic strategy. The development of CRC is a multistep process driven by multiple somatic gene mutations, such as activation of K-*ras* oncogenes and inactivation of *APC* and *p53* genes, which are involved in the pathogenesis of CRC. Assays involving molecular detection of these genetic alterations have been shown to be potential diagnostic tools for CRC by giving us a better understanding of its pathogenesis [13, 22, 23].

In the present study, we tested three genetic biomarkers for the detection of CRC in the DNA from serum samples. Our results show that 30.4%, 34.0%, and 34.2% of CRC patients with gene mutations in tumor tissues have mutated *APC,* K-*ras,* or *p53* DNA detected, respectively, in their sera; and 46.2% of patients with CRC have at least one detectable circulating genetic marker. Mutant DNA was not detected in the serum specimens of 26 patients whose tumors tested negative for genetic alterations or in the healthy subjects. This finding is consistent with the results reported by Hibi et al. [11], who showed that either a K-*ras* or a *p53* mutation was detected in the serum in 40% of the CRC patients. Our K-*ras* detection rate (34%) in the serum parallels that of other investigations, in which the incidence of mutated K-*ras* in serum or plasma of CRC patients was between 19% and 86% [23–27]. On the other hand, the circulating mutated *APC* (30.4%) or *p53* (34.2%) detection rate in our analysis was markedly different from the findings of previous reports [24, 28]. Lauschke et al. [24] and Hibi et al. [11] showed that circulating APC and p53 tumor DNA was found in 80% and 70%, respectively, of CRC tissues exhibiting gene mutations. Conversely, Gocke's group [28] reported significantly lower detection of *APC* and *p53* mutations in the plasma and serum of CRC patients than we have reported. The considerable variation in the incidence of detecting *APC*, K-*ras*, and *p53* mutations are most likely attributable to the different geographic areas, sources of specimens, time of sample collection, and the sensitivity of the analyses.

Moreover, detection of circulating tumor DNA by each molecular genetic marker and the overall detection rate showed a tendency toward a higher detection rate in CRC patients with advanced-stage disease or metastasis. Our data revealed that the overall positive tumor DNA detection rates in serum increased from 0% in Dukes' class A CRC patients to 66.7% in Dukes' class D CRC patients, which emphasizes its possible significance as a prognostic marker. Simultaneously, a higher incidence of CRC with advanced-stage disease or lymph node metastasis was identified in patients with a positive test for circulating tumor DNA in our study. Moreover, positive serum tumor DNA results correlated directly with recurrence or metastases; it therefore may be a potential prognostic indicator for CRC patients. Detection rates of disseminated cancer cells were higher in CRC patients whose tumors were at an advanced stage than in those with early-stage tumors, as proposed previously [29–31]. Furthermore, the detection of these circulating tumor cells may be of prognostic value and therefore has therapeutic implications for these patients [32]. Because of our limited follow-up period, any comment on the significance of this difference must be prefaced by information on a longitudinal follow-up of these patients. Additionally, standardization of the methods for detecting micrometastasis and disseminated tumor cells is a prerequisite for further studies.

Conclusions

The *APC*, K-*ras*, and *p53* mutations seem to be good genetic markers for detecting any circulating tumor DNA from CRCs. However, there remains a need to identify additional molecular markers, as only 75% of all CRCs harbor mutations of at least one of these three genes. The prognostic value of these genetic biomarkers for CRC patients needs further evaluation in a larger patient population with a long-term follow-up. If our results are confirmed, genetic analysis of circulating tumor DNA may have clinical applications in the future.

Résumé. La détection précoce d'ADN tumoral dans le sérum/plasma avant le développement de métastases ou des récidives pourraient contribuer à améliorer l'évolution des patients porteurs de cancer colorectal (CRC) après résection tumorale. Les progrès récents dans la détection de l'ADN tumoral dans le sérum/plasma a ouvert de nouveaux domaines d'investigation et de nouvelles possibilités de diagnostic moléculaire. Les mutations *APC* et K-*ras* sont considérées comme des évènements précoces dans le développement des cancers colorectaux, alors que les mutations *p53* sont relativement tardives dans la genese tumoro-genèse des cancers colorectaux. Le but de cette étude a été de rechercher la présence de mutations génétiques de l'ADN dans le sérum des patients porteurs de cancer colorectal et chez des sujets sains. Nous avons simultanément évalué la signification des mutations *APC*, K-*ras,* et *p53* dans les tissus cancéreux et pratiqué dans le sérum de 104 patients porteurs de cancer colorectal une analyse de polymorphisme de réction en chaîne polymérase à une conformation à simple chaîne (PCR-SSCP), suivi de séquences directes. De plus, on a cherché à détecter l'antigène carcinoembryonnaire chez les patients porteurs de cancer colorectal. Globalement, nous avons trouvé au moins une mutation génétique dans les tissus tumoraux chez 75% (78/104) des patients porteurs de cancer colorectal. La comparaison de trois marqueurs moléculaires a montré que les taux de détection dans le

sérum ont été de 30.4%, 34.0% et 34.2%, respectivement, pour les gènes *APC*, K-*ras,* et *p53*. Parmi ces patients, 46.2% (36/78) avaient un taux positif dans leur sérum alors que tous les contrôles sont restés négatifs. Les taux de détection de tumeur ADN positif dans le sérum ont été de 0% (0/7) dans les patients Dukes A, de 22.4% (11/49) chez les patients Dukes B, de 48.7% (19/39) chez les patients Dukes C et de 66.7% (6/9) chez les patients Dukes D. Le taux de détection a augmenté parallèlement au stade tumoral ($p = 0.012$). En même temps, on a noté une différence significative entre les métastases ganglionnaire lymphatique et la détection d'ADN tumoral ($p < 0.001$). On a mis en évidence également un rapport métastases postoperatoires/récidive chez les patients porteurs de mutations génétiques d'ADN tumoral par rapport aux patients sans ADN ($p < 0.001$). Cependant, aucune corrélation significative n'a été observée entre le rapport métastase postopératoire/récidive et les taux d'ACE ($p = 0.247$). Ces données suggèrent que l'identification de l'ADN tumoral circulant par la détection moléculaire de mutations des gènes d'*APC*, K-*ras*, et *p53* pourrait être un outil utile pour la détection précoce des récidives/métastases postopératoires et pourrait constituer un marqueur moléculaire d'une évolution néfaste des patients porteurs de CCR.

Resumen. La detección precoz en suero/plasma del DNA tumoral, antes de que se originen metástasis o recidivas, puede mejorar los resultados clínicos en pacientes con cáncer colorrectal (CRC) tratados quirúrgicamente. Recientes avances en la detección en plasma/suero del DNA tumoral han abierto numerosos campos para la investigación y nuevas posibilidades para el diagnóstico molecular. Se piensa que las mutaciones del *APC* y del K-ras ocurren en estadios precoces del desarrollo de los cánceres colorrectales mientras que las mutaciones de la p53 constituyen un evento relativamente tardío de la tumorogénesis del cáncer colorrectal. El objectivo de este estudio fue investigar en el suero obtenido de pacientes con cáncer colorrectal y en voluntarios sanos, la presencia de mutaciones genéticas en el DNA. Evaluamos simultáneamente en tejido neoplásico y en muestras pareadas de suero de 104 pacientes con cáncer colorrectal la importancia de las mutaciones genéticas de la *p53*, *APC*, y K-*ras*. Para ello utilizamos un análisis de conformación del polimorfismo mediante la reacción en cadena de la polimerasa (PCR-SSCP), seguido de una secuenciación directa. Además, se llevaron a cabo análisis para detectar el nivel sérico del antígeno carcinoembrionario en pacientes con CRC. En conjunto encontramos al menos una mutación genética en el 75% (78/104) de los tejidos neoplásico de pacientes con CRC. Comparando los 3 marcadores moleculares constatamos que las tasas de detección en suero fueron del 30.4% para el *APC*, 34% para el K-*ras* y del 34.2% para la p53. De estos pacientes el 46.2% (36/78) fueron identificados como sero positivos mientras que los controles en voluntarios sanos fueron negativos. En conjunto la tasa de detección positiva para el DNA tumoral en suero fue 0% (0/7) en estadio A de Duke, 22.4% (11/49) para el estadio B, 48.7% (19/36) para el C y 66.7% (6/9) para el estadio D; el porcentaje de detección pues, aumenta a medida que el estadio tumoral avanza ($p = 0.0012$). La positividad sérica en la detección del DNA tumoral se correlacionó significativamente con la existencia de metástasis ganglionares ($p < 0.001$). Demostramos también un mayor índice de recidivas/metástasis postoperatorias en pacientes cuyo suero presentaba mutaciones genéticas en el DNA tumoral que en aquellos otros sin DNA tumoral ($p < 0.001$). Sin embargo, no encontramos relación significativa alguna entre el índice de metástasis/recidivas postoperatorias con los niveles séricos de CEA ($p = 0.247$). Nuestros hallazgos sugieren que la identificatión del DNA circulante, utilizando la detección molecular de *APC*, K-*ras*, y de las mutaciones genéticas *p53* podría ser un test válido para la detección precoz en el postoperatorio de metástasis / recidivas, además de potenciales marcadores moleculares de los pobres resultados clínicos en pacientes con CRC.

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References

1. Coleman MP, Esteve J, Damiecki P, et al. Trends in cancer incidence and mortality. L.A.R.C. Sci. Publ. 1993;121:225–256
2. Boyle P, Langman JS. ABC of colorectal cancer:epidemiology. B.M.J. 2000;321:805–808
3. Byers T, Levin B, Rothenberger D, et al. American Cancer Society guidelines for screening and surveillance for early detection of colorectal polyps and cancer: update 1997; American Cancer Society Detection and Treatment Advisory Group on Colorectal Cancer. CA. Cancer J. Clin. 1997;47:154–160
4. Mandel JS, Bond JH, Church TR, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. N. Engl. J. Med. 1993;328:1365–1371
5. Portera CA, Berman RS, Ellis LM. Molecular determinants of colon cancer metastasis. Surg. Oncol. 1998;7:183–195
6. Wong IHN, Yeo W, Chan AT, et al. Quantitative relationship of the circulating tumor burden assessed by reverse transcription-polymerase chain reaction for cytokeratin 19 mRNA in peripheral blood of colorectal cancer patients with Dukes' stage, serum carcinoembryonic antigen level and tumor progression. Cancer Lett. 2001;162:65–73
7. Wong IHN, Yeo W, Chan AT, et al. Quantitative correlation of cytokeratin 19 mRNA level in peripheral blood with disease stage and metastasis in breast cancer patients: potential prognostic implications. Int. J. Oncol. 2001;18:633–638
8. Shapiro B, Chakrabarty M, Cohn EM, et al. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer 1983;51:2116–2120
9. Stroun M, Anker P, Maurice P, et al. Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology 1989;46:318–322
10. Chen XQ, Stroun M, Magnenat JL, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat. Med. 1996;2:1033–1035
11. Hibi K, Robinson CR, Booker S, et al. Molecular detection of genetic alterations in the serum of colorectal cancer patients. Cancer Res. 1998; 58:1405–1407
12. Mulcahy HE, Lyautey J, Lederrey C, et al. A prospective study of K-ras mutations in the plasma of pancreatic cancer patients. Clin. Cancer Res. 1998;4:271–275
13. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N. Engl. J. Med. 1988;319:525–532
14. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–767
15. Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. J. Med. Genet. 1989;36:801–818
16. Perucho M. Cancer of the microsatellite mutator phenotype. Biol. Chem. 1996;377:675–684
17. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1989;6.22–6.34
18. Aust DE, Terdiman JP, Willenbucher RF, et al. The APC/beta-catenin pathway in ulcerative colitis-related colorectal carcinomas: a mutational analysis. Cancer 2002;94:1421–1427
19. Lin SR, Tsai JH, Yang YC, et al. Mutations of K-ras oncogene in human adrenal tumours in Taiwan. Br. J. Cancer 1998;77:1060–1065
20. Wang JY, Lin SR, Hsieh JS, et al. Mutations of p53 gene in gastric carcinoma in Taiwan. Anticancer Res. 2001;21:513–520
21. Astler VB, Coller FA. The prognostic significance of direct extension of carcinoma of the colon and rectum. Ann. Surg. 1954;139:846–851
22. Bos JL, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. Nature 1987;327:293–297
23. Baker SJ, Fearon ER, Nigro JM, et al. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science 1989;244:217–221
24. Lauschke H, Caspari R, Friedl W, et al. Detection of APC and k-ras mutations in the serum of patients with colorectal cancer. Cancer Detect. Prev. 2001;25:55–61
25. Anker P, Lefort F, Vasioukhin V, et al. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology 1997;112:1114–1120
26. De Kok JB, van Solinge WW, Ruers TJ, et al. Detection of tumour DNA in serum of colorectal cancer patients. Scand. J. Clin. Lab. Invest. 1997;57:601–604
27. Kopreski MS, Benko FA, Kwee C, et al. Detection of mutant K-ras DNA in plasma or serum of patients with colorectal cancer. Br. J. Cancer 1997;76:1293–1299

28. Gocke CD, Benko FA, Kopreski MS, et al. p53 and APC mutations are detectable in the plasma and serum of patients with colorectal cancer (CRC) or adenomas. Ann. N. Y. Acad. Sci. 2000;906:44–50
29. Soeth E, Vogel I, Roder C, et al. Comparative analysis of bone marrow and venous blood isolates from gastrointestinal cancer patients for the detection of disseminated tumor cells using reverse transcription PCR. Cancer Res. 1997;57:3106–3110
30. Yamaguchi K, Takagi Y, Aoki S, et al. Significant detection of circulating cancer cells in the blood by reverse transcriptase-polymerase chain reaction during colorectal cancer resection. Ann. Surg. 2000;232:58–65
31. Douard R, Le Maire V, Wind P, et al. Carcinoembryonic gene member 2 mRNA expression as a marker to detect circulating enterocytes in the blood of colorectal cancer patients. Surgery 2001;129:587–594
32. Vogel I, Kalthoff H. Disseminated tumour cells: their detection and significance for prognosis of gastrointestinal and pancreatic carcinomas. Virchows Arch. 2001;439:109–117