Cellular Oncology 28 (2006) 161–166
IOS Press

KRAS, *p53* and *BRAF* gene mutations and aneuploidy in sporadic colorectal cancer progression

Daniele Calistri [a,*], Claudia Rengucci [a], Ian Seymour [a], Elena Leonardi [b], Mauro Truini [c], Davide Malacarne [c], Patrizio Castagnola [c] and Walter Giaretti [c]

[a] *Department of Medical Oncology, Morgagni-Pierantoni Hospital, Forlì, Italy*
[b] *Department of Pathology and Cytometry Laboratory, St. Chiara Hospital, Trento, Italy*
[c] *National Institute for Cancer Research, Genoa, Italy*

Abstract. *Background:* The origin and mechanisms of chromosomal instability are still widely unknown. We previously investigated a limited number of human sporadic colorectal cancers (CRCs) and observed a statistically different occurrence of *KRAS* and *p53* mutations among predetermined subgroups of tumors with different degrees of DNA aneuploidy. The aim of the present study was to further verify these observations by including *BRAF* gene analysis and by investigating a larger series of cases subdivided into Dukes' stages A to D to reconstruct some form of chronological modulation for events during CRC progression. *Methods: KRAS*, *p53*, *BRAF* mutations and flow cytometric DNA Index were evaluated by established techniques in a series of 135 human sporadic CRCs. *Results: p53*, *KRAS* and *BRAF* mutations were found in 39%, 34%, and 4% of tumors, respectively. The frequency of *p53* mutations increased from 15% for stage A to 48% for stage D and was highest in near-diploid (DI $<$ 1.4 and DI $\neq$ 1) and high-aneuploid (DI $>$ 1.6) tumors. A similar correlation between gene mutations and DI values was observed for *KRAS*. The simultaneous presence of *KRAS* and *p53* mutations was observed in only 11% of cases. Moreover, the co-occurrence of *p53* and *KRAS* mutations was only observed in near-diploid and high-aneuploid tumors. *Conclusion:* Our findings suggest that *KRAS* and *p53* gene mutations, which are rarely simultaneous and are associated with specific DI aneuploid values, do not represent a synergistic evolutionary pathway but may influence mechanisms of chromosomal instability.

Keywords: Gene mutations, aneuploidy, colorectal cancer

1. Introduction

Colorectal cancer (CRC) is characterized by a multistep accumulation of somatic mutations in tumor suppressor genes and oncogenes and, as for the large majority of solid tumors, by genomic alterations at the chromosomal level which are the bona fide consequence of chromosomal instability (CIN) [3,8,17, 24].

Losses or gains of defined chromosomal regions are observed even in colorectal adenomas of very small size, suggesting an important role of these alterations in driving tumor transformation [27]. The molecular mechanisms of CIN, especially in generating aneuploidy, seem to be associated with telomere dysfunction, mitotic checkpoint impairment, defects in cytokinesis, or centrosome amplification [17]. A large number of genes have been identified that are likely to be involved in chromosomal integrity and linked to CIN phenotype [1,10,20,31].

Chromosomal alterations seem to be important events in the multistep development of CRC that cooperate with mutations of tumor-associated master genes such as *APC*, *KRAS* and *p53* [1,6,10,12,17–20,27,31]. However, the relation between CIN and these mutations is not yet fully understood. One possible role recently proposed for CIN and aneuploidy in tumor development is their association with an increase in the mutation rate of these and other genes [8].

Conversely, mutations in these genes could contribute to the CIN phenotype, whilst also accelerating

*Corresponding author: Daniele Calistri, PhD, Department of Medical Oncology, Morgagni-Pierantoni Hospital, Via Forlanini 34, 47100 Forlì, Italy. Tel.: +39 0543 731623; Fax: +39 0543 731736; E-mail: biomolec@ausl.fo.it.

the multistep process of transformation and progression [17]. Although still controversial, this hypothesis has been put forward for *KRAS* and CIN in several studies using both *in vitro* and *in vivo* models and biopsies from colorectal adenomas [6,12]. Moreover, the vast majority of publications on *p53* show that this oncosuppressor gene, which is inactivated late in a high percentage of CRCs, is involved in the control of many cellular processes such as apoptosis, DNA repair and DNA integrity including CIN [16,26].

Previous data indicate that the simultaneous presence of *KRAS* and *p53* gene mutations in the same tumor is not a frequent event, suggesting that these mutations do not represent a synergistic evolutionary pathway in CRC [5,28].

Additionally, a recent pilot study with a small number of CRC cases showed that the frequency of *KRAS* and *p53* mutations is associated with different DI aneuploid groups, indicating that KRAS and p53 may influence CIN mechanisms [14].

Moreover, *KRAS* mutations in CRC have generally been found to be inversely correlated with *BRAF* mutations [7,11,29,33]. The *BRAF* oncogene is one of the *Raf* genes involved in the important Ras/Raf/MEK/MAP kinase intracellular signalling pathway. In sporadic CRCs with the CIN phenotype, *BRAF* mutations are rarely observed, they are much more frequent in CRCs displaying the microsatellite instability phenotype [25,32].

In the present study we investigated potential relationships between *KRAS*, *p53* and *BRAF* mutations, the degree of DNA ploidy, tumor stage and site in human sporadic CRC.

2. Methods

2.1. Case series

Samples from 135 histologically confirmed CRCs were analyzed. Patients were recruited by Forlì and Rimini Hospitals and by the National Institute for Cancer Research, Genoa, and the series was consecutive for each tumor stage. Pathological stage was defined according to Dukes' classification: 26 patients had stage A, 40 stage B, 44 had stage C and 25 had stage D disease. Furthermore, 42 cancers were proximal and 93 were distal. Of the 135 patients, 66 were male and 69 were female. Median age was 69 years (range 35–90).

2.2. Mutation analysis

p53 exons 5–8 and *KRAS* exons 1–2 alterations were detected by single strand conformation polymorphism (SSCP) analysis. The primer sequences used for mutation analysis have been previously described [21,22]. Briefly, DNA amplification was performed in a final volume of 25 μl containing 0.4 mM of each primer, 200 μM dNTPs, 1 $\times$ reaction buffer with 3.5 mM $MgCl_2$ and 1 unit of Taq polymerase (Qiagen, Hilden, Germany). The reaction mixture was subjected to 38 PCR cycles: 60 seconds at 94°C, 60 seconds at 58°C and 60 seconds at 72°C.

All mutations were confirmed by sequencing with a 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the supplier's instructions.

BRAF gene exons 11 and 15 were amplified using previously published primers [4] and directly sequenced by a 3100-Avant Genetic Analyzer (Applied Biosystems) according to the supplier's instructions.

2.3. DNA Index analysis

The degree of DNA ploidy (DNA Index, DI) was evaluated by flow cytometry according to consensus criteria [23] using suspensions of nuclei stained with 4,6-diamidino-2-phenylindole-2-hydrochloride (DAPI; Sigma Chemical, St. Louis, MO, USA). Flow cytometry was performed to evaluate three parameters: nuclear DAPI fluorescence, proportional to DNA content, and forward and perpendicular nuclear scatter signals, which reflect nuclear size and internal structure and are useful to separate inflammatory from epithelial nuclei [13]. DNA aneuploidy was subdivided into four groups: DNA diploid (DI $= 1$), near-diploid (DI < 1.4 and $\neq 1$), near-triploid (DI $= 1.5 \pm 0.1$) and high aneuploid (DI > 1.6).

DI analysis was not performed for 18 cases because of insufficient levels of starting material.

2.4. Statistical analysis

Pearson's chi-square was used to test the hypothesis of equal distribution of molecular alterations in different stages of disease. A chi-square probability of 0.05 or less was considered to be statistically significant. No adjustment was made for performing multiple tests.

3. Results

3.1. KRAS, p53, BRAF gene mutations and clinicopathological associations

Genetic alterations of *p53* and *KRAS* genes were present in a high percentage of cases (39% and 34%, respectively). Conversely, *BRAF* mutations were comparatively rare events (4%).

p53 gene mutations were localized in "hot spot" codons 175, 245, 248 and 273 in more than one-third of cases. The GC to AT transitions represented 58% of *p53* gene mutations and were localized, in the vast majority of cases, at CpG dinucleotides.

Eighty percent of *KRAS* gene alterations consisted of substitutions at codon 12, with both transition and transversion mutation types. Seven mutations, all G to A transitions, occurred at codon 13, and only two transversions were localized at codon 61.

The four *BRAF* gene mutations were represented by the common alteration found at codon V599E.

The frequency of *p53* gene mutations was significantly lower in Dukes' stage A tumors than in all other stages ($p = 0.007$) and showed a trend towards lower incidence in distal compared to proximal sites (Table 1). Conversely, no associations were observed between tumor stage or location and *KRAS* and *BRAF* mutations.

3.2. DNA Index values and clinicopathological associations

DNA aneuploid Index values (DI $\neq 1$) were detected in 75% of cases (88/117), with a similar occurrence of near-diploid and high-aneuploid subpopulations (25% and 33%, respectively) and a somewhat lower percentage of cases with near-triploid DI values (17%) (Table 2).

Table 1

p53, KRAS and *BRAF* gene mutations and histopathological characteristics

	Overall	*p53*		*KRAS*		*BRAF* (No. cases = 100)	
		No.	%	No.	%	No.	%
	135	52	39	46	34	4	4
Stage							
A	26	4	15	6	23	0	0
B	40	18	45	15	37	2	8
C	44	18	41	17	39	1	4
D	25	12	48	8	32	1	4
			$p = 0.007$				
Location							
Distal	93	32	34	15	36	2	3
Proximal	42	20	48	31	33	2	7

Table 2

DNA Index (DI) and histopathological characteristics

	Diploids	Near-diploids	Near-triploids	High-aneuploids	Total
Dukes' stage	DI = 1	DI < 1.4 and ≠1	DI = 1.5 ± 0.1	DI > 1.6	No. cases
	%	%	%	%	
All stages	25	25	17	33	117
A	48	22	9	22	23
B	24	29	21	26	34
C	17	20	20	44	41
D	16	31	16	37	19
	$p = 0.007$				
Location					
Distal	21	25	19	36	81
Proximal	33	28	11	28	36

Table 3
DNA Index and mutation status

DNA Index classes	No. cases	*p53* (%)	*KRAS* (%)	Mutation co-presence (%)	At least one gene mutation (%)
Overall	117	37	33	11	59
Diploids	29	28	21	0	48
Near-triploids	20	25	10	0	35
Near-diploids	29	45	59	28	76
High-aneuploids	39	44	36	13	67
		$p = 0.05$	$p = 0.001$		

Break-down analyses as a function of tumor stage and location showed a significantly higher percentage of DNA diploid tumors (DI = 1) in Dukes' stage A tumors (48%) compared with more advanced stages ($p = 0.007$) (Table 2). In particular, for stage A tumors the lowest frequency was observed for near-triploid DI values, whereas the other DI values were equally represented. No noticeable differences were seen in the DI distribution values of any of the other Dukes' stage tumors. Similarly, the frequency for different DI values was not significantly related to tumor site.

3.3. Association between gene mutations and DNA Index values

DNA Index analysis results and gene mutation status were simultaneously available for 117 cases. A high frequency of at least one gene mutation was observed in all DNA Index classes. The highest frequencies of *KRAS* and *p53* mutations were observed among near-diploid and high-aneuploid tumors, with a statistically significant difference with respect to the other classes (*p53*, $p = 0.05$; *KRAS*, $p = 0.001$) (Table 3). Moreover, we noted a higher co-occurrence of these two gene mutations, about one third of cases, in near-diploid tumors with respect to other subgroups in which mutations were rare (high-aneuploid) or absent.

Due to the low frequency of *BRAF* gene mutations, it was not possible to carry out any correlation analyses.

4. Discussion

There is increasing evidence that chromosomal instability (CIN), revealed by widespread cytogenetic abnormalities such as specific chromosomal gains, losses and rearrangements, plays an important role in the development and progression of human cancer together with mutations of oncogenes and tumor suppressor genes [1,6,10,13,17,20,31]. CIN is the predominant form of genomic instability in most human solid tumors and in CRC in particular [15,18,19]. Unfortunately, the relationship between gene mutations and CIN remains poorly understood.

In the present study we investigated the hypothesis of an association between *KRAS*, *p53* and *BRAF* gene mutations and CIN, as reflected by the degree of DNA ploidy (DI values) in CRC patients, using tumor stage and location as covariates. Mutation frequencies of 39%, 34%, and 4% were found for *p53*, *KRAS* and *BRAF genes*, respectively, which is within the range of those reported in other studies [2,7,11,16,25,29,30, 32,33]. The low occurrence (4%) of *BRAF* mutations clearly limited the power of the statistical tests. Interestingly, the simultaneous presence of *KRAS* and *p53* mutations was observed only in 11% of cases (13 out of 117 CRCs). According to the colorectal cancer model of Vogelstein [9], *KRAS* and *p53* alterations are expected to accumulate during the CRC progression so that their combined occurrence should, in theory, be higher that the occurrence predicted for the two parameters taken as independent events. This was not confirmed in the present series of cases, suggesting that, in the presence of one mutation, there is no pressure to select the other, as reported in previous studies [5,28].

The frequency of *p53* mutations was found to increase from Dukes' stage A onwards, whereas that of DNA diploid (DI = 1) subpopulations dramatically decreased from early through later stages. Thus, at lower Dukes' stages CRCs are still capable of maintaining a stable genome, characterized in a high number of cases (48%) by DNA diploidy (DI = 1) and a lower frequency of *KRAS* and *p53* mutations. Interestingly, we found a statistically significant association between *KRAS* and *p53* mutations and predetermined subgroups of tumors with different DNA Index values. It was observed that *p53* and, in particular, *KRAS* mutations,

were significantly associated with tumors with near-diploid and high-aneuploid DNA Index values. Moreover, it was seen that *KRAS* and *p53* double-mutated CRCs were found within these two DNA Index subgroups, whereas none was present in the DNA diploid or near-triploid groups. This suggests that multiple pathways of CRC genesis and progression may exist. Our findings would also seem to indicate that near-diploid and high-aneuploid cell subpopulations share a common CIN genetic mechanism which differs from the mechanism of near-triploidization, as previously hypothesized [13,14].

The exact mechanisms explaining such differences have yet to be defined. We believe that near-diploid and high-aneuploid tumor cell subpopulations are generated by mechanisms of asymmetric chromosomal segregation and endoreduplication that are associated with *KRAS* and *p53* mutations. Aneuploidization in the near-triploid group may, conversely, be dependent on different mechanisms that are not associated with these gene mutations such as the formation of tripolar octaploid mitosis and its division into one diploid and two near-triploid cells [6]. In other words, it thus appears that these mutations may influence CIN and aneuploidy, suggesting that both genetic mutations and large-scale genomic aberrations could cooperatively drive CRC tumor progression.

The association observed between *KRAS* and *p53* mutations and different subgroups of DNA index suggests the possible existence of multiple pathways for CRC genesis and progression, which could have potential clinical implications. In particular, the presence of DNA diploid CRCs with wild type *p53* or *KRAS*, commonly associated with Dukes' stage A, may indicate a low probability for evolution into invasive and high-stage tumors.

Further studies focused on colorectal adenomas and *in vitro* and *in vivo* mouse models are needed to clarify CIN-associated molecular mechanisms and validate these alterations as biomarkers of clinical relevance.

Acknowledgements

The Authors wish to thank Prof. Rosella Silvestrini for her invaluable scientific contribution and Gráinne Tierney for editing the manuscript. Supported by *Istituto Oncologico Romagnolo*, Forlì, *Consiglio Nazionale della Ricerca* (CNR), Rome (02.00382.ST97 and CU03.00291), and by grants from Ministero della Salute (FSN 2004), S. Paolo Oncology Program, and Regione Sicilia, Italy.

References

[1] D.G. Albertson, C. Collins, F. McCormick and J.W. Gray, Chromosome aberrations in solid tumors, *Nat. Genet.* **34** (2003), 369–376.

[2] H.J. Andreyev, A.R. Norman, D. Cunningham, J. Oates, B.R. Dix, B.J. Iacopetta, J. Young, T. Walsh, R. Ward, N. Hawkins, M. Beranek, P. Jandik, R. Benamouzig, E. Jullian, P. Laurent-Puig, S. Olschwang, O. Muller, I. Hoffmann, H.M. Rabes, C. Zietz, C. Troungos, C. Valavanis, S.T. Yuen, J.W. Ho, C.T. Croke, D.P. O'Donoghue, W. Giaretti, A. Rapallo, A. Russo, V. Bazan, M. Tanaka, K. Omura, T. Azuma, T. Ohkusa, T. Fujimori, Y. Ono, M. Pauly, C. Faber, R. Glaesener, A.F. de Goeij, J.W. Arends, S.N. Andersen, T. Lovig, J. Breivik, G. Gaudernack, O.P. Clausen, P.D. De Angelis, G.I. Meling, T.O. Rognum, R. Smith, H.S. Goh, A. Font, R. Rosell, X.F. Sun, H. Zhang, J. Benhattar, L. Losi, J.Q. Lee, S.T. Wang, P.A. Clarke, S. Bell, P. Quirke, V.J. Bubb, J. Piris, N.R. Cruickshank, D. Morton, J.C. Fox, F. Al-Mulla, N. Lees, C.N. Hall, D. Snary, K. Wilkinson, D. Dillon, J. Costa, V.E. Pricolo, S.D. Finkelstein, J.S. Thebo, A.J. Senagore, S.A. Halter, S. Wadler, S. Malik, K. Krtolica and N. Urosevic, Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study, *Br. J. Cancer* **85** (2001), 692–696.

[3] G. Auer, U. Kronenwett, U. Roblick, B. Franzén, J. Habermann, R. Sennerstam and T. Ried, Human breast adenocarcinoma: DNA content, chromosomes, gene expression and prognosis, *Cell. Oncol.* **26** (2004), 171–174.

[4] M.S. Brose, P. Volpe, M. Feldman, M. Kumar, I. Rishi, R. Gerrero, E. Einhorn, M. Herlyn, J. Minna, A. Nicholson, J.A. Roth, S.M. Albelda, H. Davies, C. Cox, G. Brignell, P. Stephens, P.A. Futreal, R. Wooster, M.R. Stratton and B.L. Weber, BRAF and RAS mutations in human lung cancer and melanoma, *Cancer Res.* **62** (2002), 6997–7000.

[5] D. Calistri, C. Rengucci, I. Seymour, A. Lattuneddu, A.M. Polifemo, F. Monti, L. Saragoni and D. Amadori, Mutation analysis of p53, K-ras, and BRAF genes in colorectal cancer progression, *J. Cell. Physiol.* **204** (2005), 484–488.

[6] P. Castagnola and W. Giaretti, Mutant KRAS, chromosomal instability and prognosis in colorectal cancer, *Biochim. Biophys. Acta* 1756 (2005), 115–125.

[7] T.L. Chan, W. Zhao, S.Y. Leung and S.T. Yuen, Cancer Genome Project: BRAF and KRAS mutations in colorectal hyperplastic polyps and serrated adenomas, *Cancer Res.* **63** (2003), 4878–4881.

[8] P. Duesberg, R. Li, D. Rasnick, A. Fabarius and R. Hehlmann, Carcinogenesis by aneuploidization, *Cell. Oncol.* **26** (2004), 181–186.

[9] E.R. Fearon and B. Vogelstein, A genetic model for colorectal tumorigenesis, *Cell* **61** (1990), 759–767.

[10] R. Fodde, R. Smits and H. Clevers, APC, signal transduction and genetic instability in colorectal cancer, *Nat. Rev. Cancer* **1** (2001), 55–67.

[11] K. Fransen, M. Klintenas, A. Osterstrom, J. Dimberg, H.J. Monstein and P. Soderkvist, Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas, *Carcinogenesis* **25** (2004), 527–533.

[12] W. Giaretti, S. Molinu, J. Ceccarelli and C. Prevosto, Chromosomal instability, aneuploidy, and gene mutations in human sporadic colorectal adenomas, *Cell. Oncol.* **26** (2004), 301–305.

[13] W. Giaretti, N. Pujic, A. Rapallo, S. Nigro, A. Di Vinci, E. Geido and M. Risio, K-ras2 G-C and G-T transversions correlate with DNA aneuploidy in colorectal adenomas, *Gastroenterology* **108** (1995), 1040–1047.

[14] W. Giaretti, T. Venesio, A. Sciutto, C. Prevosto, E. Geido and M. Risio, Near-diploid and near-triploid human sporadic colorectal cancer adenocarcinoma differ for KRAS2 and TP53 mutational status, *Genes Chrom. Cancer* **37** (2003), 207–213.

[15] M. Hermsen, C. Postma, J. Baak, M. Weiss, A. Rapallo, A. Sciutto, G. Roemen, J.W. Arends, R. Williams, W. Giaretti, A. De Goeij and G. Meijer, Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability, *Gastroenterology* **123** (2002), 1109–1119.

[16] B. Iacopetta, TP53 mutation in colorectal cancer, *Hum. Mutat.* **21** (2003), 271–276.

[17] G.J.P.L. Kops, B.A.A. Weaver and D.W. Cleveland, On the road to cancer aneuploidy and the mitotic checkpoint, *Nature Rev. Cancer* **5** (2005), 773–785.

[18] A. Leslie, N.R. Pratt, K. Gillespie, M. Sales, N.M. Kernohan, G. Smith, C.R. Wolf, F.A. Carey and R.J. Steele, Mutations of APC, K-ras, and p53 are associated with specific chromosomal aberrations in colorectal adenocarcinomas, *Cancer Res.* **63** (2003), 4656–4661.

[19] A. Leslie, A. Stewart, D.U. Baty, D. Mechan, L. McGreavey, G. Smith, C.R. Wolf, M. Sales, N.R. Pratt, R.J. Steele and F.A. Carey, Chromosomal changes in colorectal adenomas: relationship to gene mutations and potential for clinical utility, *Genes Chromosomes Cancer* **45** (2006), 126–135.

[20] F. Michor, Y. Iwasa and M.A. Nowak, Dynamics of cancer progression, *Nat. Rev. Cancer* **4** (2004), 197–205.

[21] T. Nedergaard, P. Guldberg, E. Ralfkler and J. Zeuthen, A one-step DGGE scanning method for detection of mutations in the K-, N- and H-ras oncogenes: mutations at codons 12, 13 and 61 are rare in B-cell non-Hodgkin's lymphoma, *Int. J. Cancer* **71** (1997), 364–369.

[22] C.D. O' Connell, D.H. Atha, M.C. Oldemburg, J. Tian, M. Siebert, R. Handrow, K. Grooms, L. Heisler and M. de Arruda, Detection of p53 gene mutation: analysis by single-strand conformation polymorphism and cleavase fragment length polymorphism, *Electrophoresis* **20** (1999), 1211–1223.

[23] M.G. Ormerod, B. Tribukait and W. Giaretti, Consensus report of the task force on standardisation of DNA flow cytometry in clinical pathology, *Anal. Cell. Pathol.* **17** (1998), 103–110.

[24] I. Petersen, Chromosome, ploidy and genetic imbalances of lung cancer, *Cell. Oncol.* **26** (2004), 173–174.

[25] W.S. Samowitz, C. Sweeney, J. Herrick, H. Albertsen, T.R. Levin, M.A. Murtaugh, R.K. Wolff and M.L. Slattery, Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers, *Cancer Res.* **65** (2005), 6063–6069.

[26] S. Sengupta and C.C. Harris, p53: traffic cop at the crossroads of DNA repair and recombination, *Nat. Rev. Mol. Cell. Biol.* **6** (2005), 44–55.

[27] I.M. Shih, W. Zhou, S.N. Goodman, C. Lengauer, K.W. Kinzler and B. Vogelstein, Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis, *Cancer Res.* **61** (2001), 818–822.

[28] G. Smith, F.A. Carey, J. Beattie, M.J. Wilkie, T.J. Lightfoot, J. Coxhead, R.C. Garner, R.J. Steele and C.R. Wolf, Mutations in APC, Kirsten-ras, and p53-alternative genetic pathways to colorectal cancer, *Proc. Natl. Acad. Sci. USA* **99** (2002), 9433–9438.

[29] F. Sommerer, M. Vieth, A. Markwarth, K. Rohrich, S. Vomschloss, A. May, C. Ell, M. Stolte, U.R. Hengge, C. Wittekind and A. Tannapfel, Mutations of BRAF and KRAS2 in the development of Barrett's adenocarcinoma, *Oncogene* **23** (2004), 554–558.

[30] R. Soong, B. Powell, H. Elsaleh, G. Gnanasampanthan, D.R. Smith, H.S. Goh, D. Joseph and B. Iacopetta, Prognostic significance of TP53 gene mutation in 995 cases of colorectal carcinoma. Influence of tumour site, stage, adjuvant chemotherapy and type of mutation, *Eur. J. Cancer* **36** (2000), 2053–2060.

[31] Z. Wang, J.M. Cummins, D. Shen, D.P. Cahill, P.V. Jallepalli, T.L. Wang, D.W. Parsons, G. Traverso, M. Awad, N. Silliman, J. Ptak, S. Steve Szabo, J.K.V. Willson, S.D. Markowitz, M.L. Goldberg, R. Karess, K.W. Kinzler, B. Vogelstein, V.E. Velculescu and C. Lengauer, Three classes of genes mutated in colorectal cancers with chromosomal instability, *Cancer Res.* **64** (2004), 2998–3001.

[32] L. Wang, J.M. Cunningham, J.L. Winters, J.C. Guenther, A.J. French, L.A. Boardman, L.J. Burgart, S.K. McDonnell, D.J. Schaid and S.N. Thibodeau, BRAF mutations in colon cancer are not likely attributable to defective DNA mismatch repair, *Cancer Res.* **63** (2003), 5209–5212.

[33] S.T. Yuen, H. Davies, T.L. Chan, J.W. Ho, G.R. Bignell, C. Cox, P. Stephens, S. Edkins, W.W. Tsui, A.S. Chan, P.A. Futreal, M.R. Stratton, R. Wooster and S.Y. Leung, Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia, *Cancer Res.* **62** (2002), 6451–6455.