ELSEVIER

European Journal of Cancer 41 (2005) 1649–1654

European Journal of Cancer

www.ejconline.com

The prevalence of *PIK3CA* mutations in gastric and colon cancer

Sérgia Velho [a], Carla Oliveira [a], Ana Ferreira [a], António Carlos Ferreira [a], Gianpaolo Suriano [a], Simó Schwartz Jr. [b], Alex Duval [c], Fátima Carneiro [a,d,e], José Carlos Machado [a,d], Richard Hamelin [c], Raquel Seruca [a,d,*]

[a] *Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), 4200-465 Porto, Portugal*
[b] *Molecular Pathology Program, Centre d'Investigacions en Bioquímica i Biologia Molecular CIBBIM, Passeig Vall d'Hebron 119-129, Barcelona 08035, Spain*
[c] *INSERM U434 CEPH, 75010 Paris, France*
[d] *Medical Faculty of the University of Porto, 4200-465 Porto, Portugal*
[e] *Department of Pathology, Hospital de S. João, 4200-465 Porto, Portugal*

Received 3 February 2005; received in revised form 20 April 2005; accepted 22 April 2005
Available online 1 July 2005

Abstract

A wide variety of tumours show *PIK3CA* mutations leading to increased phosphatidylinositol-3 kinase (*PI3K*) activity. We have determined the frequency of *PIK3CA* mutations in exons 9 and 20 that has previously been reported as mutational hotspot regions in distinct tumour models. One hundred and fifty gastrointestinal carcinomas (47 gastric and 103 colorectal) that were characterised for MSI status (76 MSI and 74 MSS) by PCR-SSCP sequencing were evaluated. We also analysed the association between *PIK3CA* mutations and *KRAS* or *BRAF* mutations. *PIK3CA* mutations in exons 9 and 20 were present in 13.6% and 10.6% of colorectal and gastric carcinomas, respectively. No differences in frequency and type of *PIK3CA* mutations were found between MSI and MSS colorectal carcinomas. All gastric carcinomas with *PIK3CA* mutations were MSI. The number of cases harbouring concomitant *PIK3CA* and *KRAS* or *BRAF* mutations was higher in colorectal than in gastric carcinomas ($P = 0.016$). In colorectal carcinoma, *PIK3CA* mutations occur preferentially together with activating *KRAS-BRAF* mutations (MSI and MSS) while in gastric carcinomas *PIK3CA* mutations tend to occur as isolated events (MSI).

Keywords: Genetic instability; *PIK3CA*; *BRAF*; *KRAS*; DNA mismatch repair; MSI; Gastrointestinal cancer; Gastric cancer; Colorectal cancer

1. Introduction

Phosphatidylinositol-3 kinases (PI3Ks) constitute a large and complex family of lipid kinases encompassing three classes with multiple subunits and isoforms [1–3]. PI3Ks play an important role in several cellular functions, such as proliferation, differentiation, chemotaxis, survival, trafficking, and glucose homeostasis [2]. Class IA PI3Ks are heterodimeric proteins composed by a p110 catalytic subunit and a p85 regulatory subunit [4]. p85 lacks kinase activity and acts as an adaptor, coupling the p110 subunit to activate protein tyrosine kinases [5]. This class of PI3Ks can be activated through interaction with phosphotyrosine residues of receptor tyrosine kinases (RTK) [6–9] or through the binding of active RAS to the p110 catalytic subunit [3,9–12]. Active PI3Ks phosphorylate the inositol ring 3′OH group in inositol phospholipids to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3) [13], which in turn activates diverse cellular target proteins such as the survival signalling kinase AKT/PKB [1,2,14,15]. A tumourigenic role has been proposed for

* Corresponding author. Tel.: +351 22 5570774; fax: +351 22 5570799.
E-mail address: rseruca@ipatimup.pt (R. Seruca).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.04.022

PIK3CA gene that encodes the catalytic subunit p110alpha of phosphatidylinositol 3-kinase belonging to class IA of PI3Ks [2,3,16]. Recently, mutations in *PIK3CA* were identified in different tumour models, namely colorectal cancer, gastric cancer, glioblastoma, breast and lung cancer [17]. Additionally, several other studies in hepatocellular carcinomas, breast carcinomas, lung cancer, ovarian carcinomas, brain tumours and acute leukaemias emphasised the oncogenic potential of *PIK3CA* in the development of cancer [18–21].

In the study of Samuels [17], two *PIK3CA* mutational "hotspots" were described that affected the helical (exon 9) and catalytic (exon 20) protein domains, respectively. In colon carcinomas exons 9 and 20 were preferentially mutated in *PIK3CA*. Further, in colon carcinomas *PIK3CA* mutations were also described in exons 1, 2, 4, 7, 12, 14 and 18, but these mutations only occurred in a minority of cases [17,18]. Similar to colon tumours, *PIK3CA* mutations clustered in the two hotspot regions (exons 9 and 20) in gastric carcinomas also [17,19,22]. In a single case of gastric carcinoma, exon 18 coding for *PIK3CA* was also reported to be mutated however, this result was not confirmed when a larger series of tumors was analysed [17,22].

Although *PIK3CA* mutations have been already reported in several tumour types, the mutation frequency of this gene in gastrointestinal carcinoma is still controversial. Furthermore, very little is known about the frequency of *PIK3CA* mutations within the context of gastrointestinal carcinomas with mismatch repair deficiency. It is well known that 15% of sporadic gastrointestinal carcinoma have mismatch repair deficiency leading to hundreds of thousands of mutations in microsatellite sequences throughout the genome (MSI phenotype), mainly due to the silencing of *hMLH1* through promoter methylation [23–27]. In MSI gastrointestinal carcinoma, mutations occur both in non-coding and coding sequences. Several genes involved in signalling pathways, such as the Wnt/APC/beta-catenin pathway, the TGFbeta pathway, and the MAPKinase pathway are also prone to mutation [28]. One of the best-studied pathways in MSI and microsatellite stable (MSS) sporadic gastrointestinal carcinomas is the RAS/RAF/MAPKinase pathway, in which *KRAS* or *BRAF* are activated by mutations [29–37]. Moreover, it has been demonstrated that *KRAS* and *BRAF* genes are differently involved in the development/progression of MSI and MSS colorectal and gastric carcinomas [30,31].

In the present study, we have aimed to: (a) determine the frequency of *PIK3CA* mutations at the two hotspot regions of the gene (exons 9 and 20) in a series of 150 gastrointestinal carcinomas (47 gastric and 103 colorectal) previously characterised for MSI status (76 MSI and 74 MSS) and (b) analyse the relationship between mutations in *PIK3CA* and *KRAS* or *BRAF* genes.

2. Material and methods

2.1. Tissue specimens

A total of 103 sporadic colorectal cancers (50 MSI and 53 MSS), 47 sporadic gastric cancers (26 MSI and 21 MSS) were screened for *PIK3CA* mutations in the two hotspot exons (9 and 20).

2.2. Mutations screening

For exon 9, the screening was done by PCR-SSCP-automated sequencing. The set of primers used to amplify this exon was 5′-GCT TTT TCT GTA AAT CAT CTG TG-3′ (sense sequence) and 5′-CTG AGA TCA GCC AAA TTC AGT-3′ (antisense sequence). The annealing temperature was 59 °C and PCR products were loaded into 0.6× MDE gels after denaturation and run at 20 °C overnight. Cases showing aberrant bands in comparison with a normal control were submitted to a second independent PCR. PCR products were purified and sequenced on an ABI Prism 377 automated sequencer (Perkin–Elmer, Foster City, CA) using the ABI prism dye terminator cycle sequencing kit (Perkin–Elmer) and the original primers.

Exon 20 was analysed by PCR-automated sequencing. This exon was divided in two overlapping fragments. The sets of primers used to amplify the two amplicons were: 5′-CAT TTG CTC CAA ACT GAC CA-3′ (sense sequence), 5′-TAC TCC AAA GCC TCT TGC TC-3′ (antisense sequence) for the first part of the exon (T_m = 56 °C) and 5′-ACA TTC GAA AGA CCC TAG CC-3′ (sense sequence), 5′-CAA TTC CTA TGC AAT CGG TCT-3′ (antisense sequence) for the second part of the exon (T_m = 56 °C). PCR products were loaded in 2% agarose gels, purified and sequenced as previously for exon 9.

2.3. Statistical analysis

Associations between *PIK3CA* mutations, the MSI status of the tumours and the presence of *BRAF* and/or *KRAS* mutations were assessed by the χ^2 test. A P value $\leqslant 0.05$ was considered to be statistically significant.

3. Results

From the 150 gastrointestinal carcinomas analysed, *PIK3CA* mutations in exons 9 or 20 were detected in 12.7% (19/150) of the cases. Examples of mutations are shown in Fig. 1.

The frequency of *PIK3CA* mutations was 13.6% (14/103) in colorectal carcinomas and 10.6% (5/47) in gastric carcinomas (P = 0.61). In colorectal carcinomas, the frequency of mutations was similar (P = 0.49) in MSI (16%

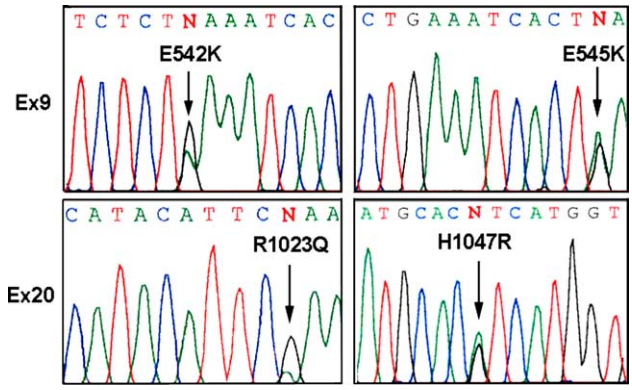

Fig. 1. Examples of *PIK3CA* mutations affecting exons 9 or 20 detected in a series of 150 gastrointestinal tumours.

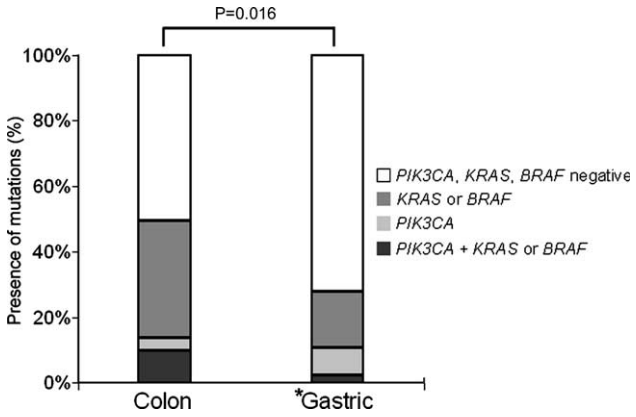

Fig. 2. Graphic representation of the relationship between concomitant *PIK3CA* mutations and *KRAS* or *BRAF* mutations; *PIK3CA* mutations alone; *KRAS* or *BRAF* alone; and cases without mutations in these three genes in colorectal and gastric carcinomas. *, In gastric carcinoma 7/47 carcinomas show *KRAS* mutations and only 1/47 display a *BRAF* mutation.

(8/50)) and MSS (11.3% (6/53)) tumours. In gastric carcinomas, a significant difference ($P = 0.03$) was observed in the frequency of *PIK3CA* mutations in MSI (19.2% (5/26)) and MSS (0% (0/21)).

In the whole series, we identified 19 samples in which mutations had occurred and they were evenly distributed in the two hotspot *PIK3CA* gene regions (Table 1). Eleven (57.9%) mutations clustered within exon 9 and eight (42.1%) mutations within exon 20. Distribution of mutations within the two hotspots was similar in colorectal and gastric carcinomas. No differences were observed when the two subsets of colorectal carcinoma (MSS and MSI) or the MSI gastric carcinomas were considered. The distribution of the mutations and amino acid changes are shown in Table 1.

The number of cases harbouring concomitant *PIK3CA* and *KRAS* or *BRAF* mutations was higher in colorectal carcinomas (9.7% (10/103)) than in gastric carcinomas (2.1% (1/47)) ($P = 0.016$) (Fig. 2). Further, we evaluated the relationship between *PIK3CA* mutations and the presence of mutations in *KRAS* or *BRAF* oncogenes in the three distinct subsets of carcinomas that showed *PIK3CA* mutations (MSI and MSS colorectal carcinomas and MSI gastric carcinomas). MSS gastric carcinomas were not considered for this analysis since mutations in *PIK3CA* were not identified in this group of tumours. Overall, in our series of colorectal carcinomas, *PIK3CA* mutations were significantly more frequent in cases harbouring mutations in *KRAS* or *BRAF* genes than in cases negative for *KRAS* and *BRAF* mutations ($P = 0.037$). From a total of 47 colorectal carcinomas with *KRAS* or *BRAF* mutations, 21.3% of the cases (10/47) had concomitant *PIK3CA* mutations. In contrast, only 7.1% (4/56) of the colorectal tumours lacking *KRAS* or *BRAF* mutations had mutations in *PIK3CA*. Within colorectal carcinomas, concomitant mutations at *PIK3CA* and *KRAS* or *BRAF* genes occurred both in MSI and MSS tumours. The frequency of concomitant *PIK3CA* and *KRAS* or *BRAF* genes was 10.0% (5/50) in MSI and 9.4% (5/53) in MSS colorectal carcinomas (Table 2). In gastric carcinomas, both-*PIK3CA* and *KRAS* mutations occurred exclusively in MSI tumours. In this group of tumours, 4 of the 5 (80%) *PIK3CA* mutations occurred in a *KRAS* wild type background (Table 2). In MSI gastric carcinomas *BRAF* mutations were not identified. One *BRAF* mutation was found in a MSS gastric carcinoma (Table 2).

Table 1
Number of cases with mutations of *PIK3CA* and amino acid substitutions in gastrointestinal tumours

	Amino acid substitution	MSI colorectal cancer ($n = 50$)	MSS colorectal cancer ($n = 53$)	MSI gastric cancer ($n = 26$)	Total cases
Exon 9	E542K	3	2	3	8
	E545K		2		2
	E545G	1			1
Exon 20	R1023Q	1			1
	H1047L	1			1
	H1047R	2	2	2	6

4. Discussion

Recently, much attention has been given to the role of *PIK3CA* gene mutations in several human tumours. Mutational analysis of *PIK3CA* gene has revealed that genetic alterations at its locus occur in a wide spectrum of human neoplasias. *PIK3CA* mutations preferentially occur in exons 9 and 20, affecting the two functionally important helical and kinase domains of the protein [17–22]. In colon and gastric carcinoma, it has been demonstrated that mutations of *PIK3CA* also occur preferentially in these two exons [17–19,22]. Taking these results into account, we decided to analyse further

Table 2
Association studies between *PIK3CA* mutations and mutations in *KRAS* or *BRAF* oncogenes in colorectal and gastric carcinomas

PIK3CA (exons 9 and 20)	*KRAS* or *BRAF* Mutant	*KRAS* and *BRAF* Wild-type	*P* value
Colorectal carcinomas $n = 103$			
Mutant	10	4	0.037
Wild-type	37	52	
MSI colorectal carcinomas $n = 50$			
Mutant	5	3	0.031
Wild-type	18	24	
MSS colorectal carcinomas $n = 53$			
Mutant	5	1	0.072
Wild-type	19	28	
Gastric carcinomas $n = 47$			
Mutant	1	4	ns
Wild-type	8	34	
MSI gastric carcinomas $n = 26$			
Mutant	1	4	ns
Wild-type	7	14	
MSS gastric carcinomas $n = 21$			
Mutant	0	0	–
Wild-type	1[a]	20	

ns, not statistically significant.
[a] One *BRAF* mutation.

exons 9 and 20 as they are reported hotspot regions for *PIK3CA* mutations. In this study, we report the presence of *PIK3CA* mutations in 12.7% of 150 gastrointestinal carcinomas. These mutations were evenly distributed in the two hotspot regions of the gene (exon 9 and 20). No differences were found in the frequency of *PIK3CA* mutations between colorectal and gastric carcinomas, suggesting that *PIK3CA* activation is likely to play a role in the development/progression of both tumour types.

In agreement with previous reports, 10.6% of the gastric tumours herein studied harboured *PIK3CA* mutations. Lee [19] and Samuels [17] described *PIK3CA* mutations in 6.5% and 16.7% of gastric tumours, respectively. Most of these mutations clustered within exon 20. In contrast to both reports, we did not find a particular accumulation of *PIK3CA* mutations in exon 20. We identified two *PIK3CA* mutations in codon 1047 of exon 20 and three mutations in codon 542 of exon 9. One of the exon 9 mutations in codon 542 is novel and has not been previously described in gastric cancer [17,19]. In colorectal carcinomas, previous studies have reported the prevalence of *PIK3CA* exon 9 and exon 20 mutations to range between 15% and 27% [17,18]. In this study, the frequency of *PIK3CA* mutations in colorectal tumours (13.6%) was close to those previously reported [17,18]. In agreement with recent data on this type of tumour, we observed a similar frequency of mutations in exons 9 and 20, for which codons 542, 545 and 1047 were preferentially mutated. In the above studies, codon 545 showed the highest frequency of mutation (47%). In contrast, in our series, codons 542 and 1047 were the most frequently mutated. Furthermore, we found a novel mutation of *PIK3CA* localised in codon 1023.

In the whole series of colorectal and gastric carcinomas presented here, *PIK3CA* amino acid substitutions E542K, E545K and H1047R represent the most common mutations in both tumour types. These amino acid changes were previously reported in colorectal, breast, ovarian, lung carcinomas and in brain tumours [17–21], suggesting that these mutations are likely to induce *PIK3CA* activation and thus contribute to the development of a large spectrum of tumours. In addition, these amino acid substitutions were demonstrated *in vitro* to induce significantly higher lipid kinase activity than the wild-type *PIK3CA* protein [17,38]. Further, it was demonstrated that an increase in the lipid kinase activity of the p110 alpha subunit of PI3K leads to activation of AKT and induces oncogenic transformation in cultures of chicken embryo fibroblasts [38,39]. The other amino acid substitutions E545G, R1023Q and H1047L found in few colorectal cancer samples were localised to the helical and kinase domains of *PIK3CA* protein, suggesting that they may convey selected advantage to the cancer cell. However, the effect on the enzymatic activity or the transforming potential conferred by these mutations still needs to be elucidated.

In this study, we also analysed the relationship between the presence of *PIK3CA* mutations and MSI status of the tumours. We demonstrated for the first time, that *PIK3CA* mutations cluster within the MSI subset of gastric tumours (19.2%). No single MSS gastric carcinomas bore *PIK3CA* mutations. It is likely that *PIK3CA* mutations do not represent an important oncogenic event for the development/progression of MSS gastric carcinomas, analogously to what is observed for *KRAS* and *BRAF* mutations in mismatch repair proficient gastric tumours [30,32,36]. In contrast to what was observed in stomach cancer, colorectal carcinomas harboured *PIK3CA* mutations in both MSI and MSS tumour subsets, similarly for *KRAS* mutations but not for *BRAF* mutations [30,33–35,37]. In keeping with previous data, *PIK3CA* mutations were detected within the MSI and MSS colorectal carcinomas [17], suggesting that *PIK3CA* mutations play a role in the development/progression of both subsets of colorectal carcinomas. It is well known that *KRAS–BRAF*-MAPKinase pathway is involved in sporadic MSI gastric and in sporadic MSI and MSS colorectal carcinomas. In MSI gastric carcinomas *KRAS* mutations are detected in about 30% of cases while *BRAF* mutations were have never been found [30,32]. In MSI colorectal carcinomas, *KRAS* mutations are inversely associated to *BRAF* mutations, the latter being the most frequent [30,31]. In MSS colorectal carcinomas *KRAS* mutations are very common while *BRAF* mutations are identified in only 6% of cases [29,35,37,40]. The accumulation of *PIK3CA* and *BRAF*

and/or *KRAS* mutations within colorectal and gastric carcinomas was significantly different. In our series of MSI and MSS colorectal carcinomas, *PIK3CA* mutations occurred more frequently in accumulation with *KRAS* or *BRAF* mutations than in isolation, suggesting a possible synergistic effect in the signalling pathways controlled by these genes in colorectal cancer development/progression. In contrast, in gastric carcinomas, both *PIK3CA* and *KRAS* mutations are restricted to MSI and occurred more as isolated genetic events. This observation raises the hypotheses that both *PIK3CA* and *KRAS* work as putative alternative oncogenic events in MSI gastric carcinoma development. A bigger series of MSI gastric cancer is needed to investigate this hypothesis further.

Ethics approval statement

The study protocol was reviewed and approved by the appropriate Ethics Committees. Tumour samples were obtained with informed consent from the institutions that contributed with material to the study.

Conflict of interest statement

None declared.

Acknowledgements

This study was funded by Fundação para a Ciência e a Tecnologia, Portugal (Project POCTI/SAU-OBS/56921/2004) and by Programa Operacional Ciência, Tecnologia e Inovação (POCTI), do QCA III.

References

1. Domin J, Waterfield MD. Using structure to define the function of phosphoinositide 3-kinase family members. *FEBS Lett* 1997, **410**, 91–95.
2. Katso R, Okkenhaug K, Ahmadi K, et al. Cellular function of phosphoinositide 3-kinases: implications for development, immunity, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 2001, **17**, 615–675.
3. Vivanco I, Sawyers CL. The phosphatidyllinositol 3-kinase-AKT pathway in human cancer. *Nat Rev Cancer* 2002, **2**, 489–501.
4. Carpenter CL, Duckworth BC, Auger KR, et al. Purification and characterization of phosphoinositide 3-kinase from rat liver. *J Biol Chem* 1990, **265**, 19704–19711.
5. Hiles ID, Otsu M, Volina S, et al. Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. *Cell* 1992, **70**, 419–429.
6. Carpenter CL, Auger KR, Chanudhuri M, et al. Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. *J Biol Chem* 1993, **268**, 9478–9483.
7. Porter AC, Vaillancourt RR. Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. *Oncogene* 1998, **17**, 1343–1352.
8. Wymann MP, Pirola L. Structure and function of phosphoinositide 3-kinases. *Biochim Biophys Acta* 1998, **1436**, 127–150.
9. Vanhaesebroeck B, Waterfield MD. Signaling by distinct classes of phosphoinositide 3-kinases. *Exp Cell Res* 1999, **253**, 239–254.
10. Kodaki T, Woscholski R, Hallberg B, et al. The activation of phosphoinositol 3-kinase by Ras. *Curr Biol* 1994, **4**, 798–806.
11. Rodriguez-Viciana P, Warne PH, Dhand R, et al. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* 1994, **370**, 527–532.
12. Rodriguez-Viciana P, Warne PH, Vanhaesebroeck B, et al. Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutations. *EMBO J* 1996, **15**, 2442–2451.
13. Whitman M, Downes CP, Keeler M, et al. Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* 1988, **332**, 644–646.
14. Khwaja A, Rodriguez-Viciana P, Wennstrom S, et al. Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/AKT cellular survival pathway. *EMBO J* 1997, **16**, 2783–2793.
15. Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* 2000, **346**, 561–576.
16. Volinia S, Hiles I, Ormondroyd E, et al. Molecular cloning, cDNA sequence, and chromosomal localization of the human phosphatidylinositol 3-kinase p110 alpha (PIK3CA) gene. *Genomics* 1994, **24**, 472–477.
17. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004, **304**, 554.
18. Campbell IG, Russel SE, Choong DY, et al. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res* 2004, **64**, 7678–7681.
19. Lee JW, Soung YH, Kim SY, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene* 2005, **24**(8), 1477–1480.
20. Broderick DK, Di C, Parret TJ, et al. Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. *Cancer Res* 2004, **64**, 5048–5050.
21. Bachman KE, Argani P, Samuels Y, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther* 2004, **3**, 772–775.
22. Li VS, Wong CW, Chan TL, et al. Mutations of PIK3CA in gastric adenocarcinoma. *BMC Cancer* 2005, **23,5**(1), 29.
23. Ionov Y, Peinado MA, Malkhosyan S, et al. Ubiquitous somatic mutations in simple repeated sequences reveals a new mechanism for colonic carcinogenesis. *Nature* 1993, **363**, 558–561.
24. Philp AJ, Phillips WA, Rockman SP, et al. Microsatellite instability in gastrointestinal tract tumors. *Int J Surg Investig* 2000, **2**, 267–274.
25. Toyota M, Itoh F, Imai K. DNA methylation and gastrointestinal malignancies: functional consequences and clinical implications. *J Gastroenterol* 2000, **35**, 727–734.
26. Simpson AJ, Caballero OL, Pena SD. Microsatellite instability as a tool for the classification of gastric cancer. *Trends Mol Med* 2001, **7**, 76–80.
27. Deng G, Peng E, Gum J, et al. Methylation of hMLH1 promoter correlates with the gene silencing with a region-specific manner in colorectal cancer. *Br J Cancer* 2002, **86**, 574–579.
28. Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers. *Cancer Res* 2002, **62**, 2447–2454.
29. Samowitz WS, Holden JA, Curtin K, et al. Inverse relationship between microsatellite instability and K-ras and p53 gene alterations in colon cancer. *Am J Pathol* 2001, **158**, 1517–1524.
30. Oliveira C, Pinto M, Duval A, et al. BRAF mutations characterize colon but not gastric with mismatch repair deficiency. *Oncogene* 2003, **22**, 9192–9196.

31. Kim IJ, Park JH, Kang HC, et al. Mutational analysis of BRAF and K-ras in gastric cancers: absence of BRAF mutations in gastric cancers. *Hum Genet* 2003, **114**, 118–120.
32. Brennetot C, Duval A, Hamelin R, et al. Frequent Ki-ras mutations in gastric tumors of the MSI phenotype. *Gastroenterology* 2003, **125**, 1282–1290.
33. Domingo E, Espin E, Armengol M, et al. Activated BRAF targets proximal colon tumors with mismatch repair deficiency and MLH1 inactivation. *Genes Chrom Cancer* 2004, **39**, 138–142.
34. Deng G, Bell I, Crawley S, et al. BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. *Clin Cancer Res* 2004, **10**, 191–195.
35. Koinuma K, Shitoh K, Miyakura Y, et al. Mutations of BRAF are associated with extensive hMLH1 promoter methylation in sporadic colorectal carcinomas. *Int J Cancer* 2004, **108**, 237–242.
36. Zhao W, Chan TL, Chu K, et al. Mutations of BRAF and KRAS in gastric cancer and their association with microsatellite instability. *Int J Cancer* 2004, **108**, 167–169.
37. Hasegawa A, Fukushima T, Mashiko M, et al. Alterations of the K-ras and p53 genes and microsatellite instability in sporadic colorectal carcinomas. *Anticancer Res* 2004, **24**, 2047–2052.
38. Kang S, Bader AG, Vogt PK. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA* 2005, **102**, 802–807.
39. Mizoguchi M, Nutt CL, Mohapatra G, et al. Genetic alterations of phosphoinositide 3-kinase subunit genes in human glioblastomas. *Brain Pathol* 2004, **14**, 372–377.
40. Oliveira C, Westra JL, Arango D, et al. Distinct patterns of KRAS mutations in colorectal carcinomas according to germline mismatch repair defects and hMLH1 methylation status. *Hum Mol Genet* 2004, **13**, 2303–2311.