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Higher prevalence of KRAS mutations in colorectal cancer in Saudi Arabia: Propensity for lung metastasis

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Abstract KRAS mutation is widely accepted as a key factor in colorectal tumorigenesis. Although KRAS mutation is widely studied in CRC limited data are available about mutation rates and spectrum in CRC from developing countries like Saudi Arabia where epidemiological features of the disease are different. We studied retrospectively tumor samples of 83 Saudi metastatic CRC patients for KRAS mutations in codon 12 and codon 13, to evaluate the relevance of KRAS mutation positive colorectal cancers with metastatic sites. KRAS mutation was observed in 42.2% (35/83) patients with CRC. The most common mutations were in codon 12 (p.G12D, 46%; 16/35, $P < 0.0001$), codon 12 (pG12V, 31%; 11/35, $P < 0.0001$), and codon 13 (p.G13D, 11%; 4/35, $P < 0.016$). Of these 51% and 23% of the tumors are from the left hemicolon and rectum respectively, 83% were moderately differentiated and 86% were invasive adenocarcinoma. Observed mutations are 74% in patients with advanced stage CRC ($P = 0.006$). Among patients with KRAS mutated CRC (CRC) isolated lung and liver metastases were 32% and 23% whereas in WT KRAS was 3% and 53.1% ($P < 0.005$) respectively. The study revealed 69% and 81% of colorectal patients that responded to treatment with complete response (CR)/partial response (PR)/stable disease (SD) were KRAS mutated and WT KRAS respectively ($P = 0.182$). In the mutated KRAS cohort 31% had disease progression compared to 19% in WT KRAS ($P = 0.182$). Multivariate logistic regression analysis showed WT CRC associated with 3-fold increase in positive response to first-line treatment with an odds ratio of 2.83; 95% CI 0.910–8.832. The frequency of KRAS

Abbreviations: 5FU, Fluorouracil; AJCC, American Joint Committee on Cancer; BRAF, v-rafmurine sarcoma viral oncogene homolog B1; CR, complete response; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; GDP, guanosinediphosphate; GTP, guanosine triphosphate; *KRAS* WSC, KRAS wild-type suppressor compound; *KRAS*, Kristen Rat Sarcoma; *MAPKs*, mitogen-activated protein kinases; mCRC, metastatic colorectal cancer; MSI, microstellate instability; OS, overallsurvival; PD, progressive disease; PR, partial response; RAF, serine/threonine kinase; RECIST, response evaluation criteria in solid tumors; RR, relative risk; SD, stable disease; WT, wild-type.

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mutations appears higher in the Saudi population. KRAS mutated CRC patients had a higher propensity for lung metastases by passing liver metastases indicating the need for more extensive chest imaging for effective staging. KRAS WT responds better to treatment compared to KRAS mutated colorectal cancers.

1. Introduction

Colorectal cancer (CRC) is the second most common cancer in developed countries, only surpassed by prostate cancer in men and breast cancer in women, and accounts for about 1 million new cases in 2002 and 530,000 deaths every year.[1] In Saudi Arabia it is the commonest cancer among males (11.8%) and third among females (8.8%) superseded only by breast and thyroid cancers.[2] Surgery is still the only curative treatment for patients with colorectal cancer, but chemotherapy plays an important role in prolonging disease free and overall survival of patients with CRC.[3]

The Kristen Rat Sarcoma (*KRAS*) gene encodes a signal transduction protein, which in its active state forms a complex with a guanosine triphosphate (GTP) group. This complex is inactivated by the hydrolysis of GTP to guanosinediphosphate (GDP). The frequency of mutations in the *KRAS* gene in sporadic CRC is 30–50%.[4,5] It can be as high as 90% in pancreatic cancer mostly in codon 12.[6] The most common mutations found in CRC are in exon 2 and to a lesser magnitude in exon 3.[5] If *KRAS* is mutated, the resulting complex is less sensitive to hydrolysis, remaining in a constitutively active state, leading to cell proliferation by a variety of signaling pathways, including the mitogen-activated protein kinases (*MAPKs*) pathway.[6,7] A high frequency of mutations in this gene in benign lesions suggests that, although providing a selective growth advantage to cells, it is not sufficient by itself to trigger tumor genesis. The accumulation of mutations in this gene and others, including the *APC* gene, will presumably give a selective advantage to the mutated cells, resulting in their clonal proliferation.[8] When *KRAS* is activated, it induces the *MAPK* signal transduction cascade, transferring signals from the cell membrane to the nucleus. The proteins encoded by the *RAS* gene activate *RAF* family proteins of transcription factors. The activation of these transcription factors leads to the expression of proteins that control the cell cycle.[9–11]

The addition of biologic agents that target specific signaling pathways involved in colon tumorgenesis significantly improved the response rate (RR) and overall survival (OS). However, 40–50% of mCRC patients neither show clinical benefit nor suffer from severe toxicity. Therefore, the quest for molecular markers that could predict the response to biological agent and improve clinical benefit began. The *KRAS* gene was one of the first studied due to its involvement in CRC carcinogenesis. The first retrospective studies that evaluated *KRAS* mutation status in patients treated with cetuximab or panitumumab revealed a significant association of favorable response in patients with *KRAS* WT, with RRs of 17–48%, but no response in patients with *KRAS* mutation.[12–18] The CRYSTAL and the OPUS clinical trials were the first to prospectively evaluate *KRAS* mutational status and clinical response to cetuximab.[19,20] CRYSTAL trial patients were treated with FOLFIRI alone or FOLFIRI + Cetuximab, whereas OPUS trial patients were treated with FOLFOX alone or FOLFOX + Cetuximab. Substantial association was observed between response to Cetuximab treatment and *KRAS* WT status in both studies, with no benefit seen in patients who had a *KRAS* mutation. This association was observed both in RR and progression-free survival. With this evidence, the American Society of Clinical Oncology recommended *KRAS* mutation testing in patients who are candidates of anti-EGFR therapy.[21]

In Venezuela Estrada et al.[22] reported mutations in codons 12 and 13 of the *KRAS* gene in 23.33% of patients. Of these, 28.57% were in codon 12, 57.14% were in codon 13 and 14.29% in both codons. They were more frequent in tumors located in the left hemicolon and most of them were well differentiated adenocarcinomas (58.70%) and mucinous (28.57%). The identified mutations were more frequent in Dukes C2 stage of CRC.

In Netherland, 37% (271/737) of CRC patients had *KRAS* mutation at exon 1. The predominant mutations are G > A transitions and G > T transversions, and codons 12 and 13 are the most frequently affected codons. Patients with a rectal tumor were found to have the highest frequency of G > T transversions as compared with patients with a colon or rectosigmoid tumor. This difference appeared to be confined to women with a rectal tumor harboring G > T transversions. The equal distribution of *KRAS* mutations among cases with or without a family history of colorectal cancer debates against an important role for this mutation in hereditary colorectal cancer, and could imply that *KRAS* mutations involving environmental mechanism rather than familial.[23]

In USA, Minoo et al.[24] suggested that colorectal cancer (CRC) should be viewed as heterogeneous disease, in his study 399 patients were evaluated for clinicopathological and molecular profile including *KRAS*, *BRAF* and *MSI* status. Proximal tumors showed significantly larger size, higher T-stage, more mucinous differentiation and high grade. There were high frequency of *BRAF* mutations and *MSI*-high phenotype in proximal colon cancers. Data were supporting the concept that proximal and distal CRCs are distinct pathological entities.

An Indian study[25] was done to find out *KRAS* gene mutation in CRC patients among the Kashmiri population. In a sample of 53 patients 12 had *KRAS* mutations (22.64%). *KRAS* mutation was significantly associated with advanced Duke stage ($P < 0.05$) and positive lymph node ($P < 0.05$). Moreover codon 12 *KRAS* mutations were associated with mucinous histotype ($P < 0.05$).

The aim of the current study is to evaluate the prevalence of *KRAS* mutation among the Saudi population treated at the King Fahad Medical City and try to correlate it with other clinicopathological factors and response to treatment.

2. Methods

2.1. Study population

The study was approved by the institutional Review Board. A total of 83 samples of referred colorectal cancer tumor tissue specimens were utilized in this study. Medical records and medical database of these patients were investigated and relevant data were retrieved for analysis. The data retrieved include: age, date of diagnosis, gender, stage of disease as determined by the American Joint Committee on Cancer (AJCC), metastatic sites and response to treatment.

2.2. Tissue samples and mutation analyses

Tumor tissue embedded in paraffin was collected and sent to Biomnis lab in Lyon, France (LCD-Array)[26] for the detection of mutations within codons 12 and 13 of *Kras* exon 2. The mutations tested were: p.G12S, p.G12R, p.G12C, p.G12D, p.G12A, p.G12V, p.G13D, p.G13R. The *KRAS* LCD-array kit for the detection of mutations in codon 12 and 13 of the human *KRAS* gene is based on the amplification of a short PCR fragment spanning both codons, and the subsequent identification of point mutations in codon 12 and 13 by amplicon hybridization to immobilize the capture probes. Biotin labeling of the generated 170 bp PCR fragment occurs during PCR amplification. Following a short hybridization to WT and mutation specific capture probes immobilized on the surface of the LCD-Array, bound PCR fragments are visualized using the sensitive streptavidin-enzyme-substrate cascade. To detect even small amounts of mutated *KRAS* sequences within an excess amount of WT background, amplification will be carried out in the presence of the *KRAS* WT suppressor compound (*KRAS* WSC). This molecule preferentially suppresses WT sequence amplification and therefore allows sequence-specific detection of the smallest amounts of *KRAS* mutations in codon 12 and 13.

2.3. Evaluation of response to chemotherapy

Tumor response was evaluated by CT after 12 and 24 weeks of first-line chemotherapy with 5FU/leucovorin or 5FU/leucovorin/Oxaliplain or 5FU/leucovorin/Irinotecan in mCRC. Response evaluation criteria in solid tumors (RECIST) were used to classify tumor response in complete response CR, partial response PR, stable disease SD and progressive disease (PD).[27] The PFS was calculated from the commencement of chemotherapy to either progression of disease, death from any cause or last radiological assessment.

2.4. Statistical analysis

The overall frequency of *KRAS* mutations as well as the type of mutation and affected codon will be computed for all cases with respect to age at diagnosis, gender, tumor anatomical sub-localization, AJCC stage and histological differentiation. A difference in mean values of age at diagnosis as a continuous variable was evaluated using the Student's *t*-test. Differences in the categorical variables such as gender, tumor anatomical sub-localization, AJCC stage and tumor differentiation between patients without and with *KRAS* mutations were evaluated for significance with the chi-square and Fisher's Exact tests. A *P*-value of 0.05 or less is considered statistically significant. Multivariate logistic regression model is used to determine the prognostic value of *KRAS* mutation after adjusting baseline variables. Statistical analysis was performed using the SPSS software.

3. Results

The median age of patients was 55 years (ranging from 26 to 90 years). Majority of the patients were between 40 and 60 years of age (47%). Male to female ratio was 1.3:1. Invasive adenocarcinoma was the dominant histological type with 83% and 87% were advanced stage Dukes C and D. (Table 1). *KRAS* mutation was observed in 42.2% (35/83) patients with colorectal cancer (Table 1).

Of all cases investigated 42.2% showed mutations in exon 2 of codon 12 and codon 13 of the *KRAS* gene. Conversely, 57.8% showed significant WT sequence (Fisher's test; $P < 0.0001$). The most common mutations were glycine to aspartate on codon 12 (p.G12D, 45.7% of all mutated tumors; 16 of 35 ($P < 0.0001$), glycine to valine on codon 12 (pG12V; 31.4 of mutated tumors; 11 of 35; $P < 0.0001$), and glycine to aspartate on codon 13 (p.G13D, 11.4% of mutated tumors; 4 of 35, ($P < 0.016$). These three types account for 88.5% of all

Table 1 Baseline characteristics of patients with colorectal cancer.

Characteristics	Value	
	N	%
Total number of patients	83	
Age, median (range)	55 (Range: 26–90 years)	
20–40 years	15	18
40–60 years	39	46.9
60–80 years	27	32.5
80–90 years	2	2.4
Gender (male/female)	48/35	57.8/42.2
Histologic Grade		
1	7	8.4
2	68	82
3	8	9.6
Histologic Type		
Invasive adenocarcinoma	69	83.1
Mucinous adenocarcinoma	13	15.6
Primary tumor site (Colon/rectum)	63/20	76/24
Primary stage (AJCC/Duke's classification)		
Stage I/Duke's A	3	3.6
Stage II A/Duke's B	5	6
Stage II B/Duke's B	3	4.8
Stage III A/Duke's C	6	7.2
Stage III B/Duke's C	7	8.4
Stage III C/Duke's C	2	2.4
Stage IV A/Duke's D	25	30.1
Stage IV B/Duke's D	32	38.5
No. patients with KRAS mutation	35	42.2

mutations (Table 2). Other types of mutations were less frequent.

Of the studied patients 51.4% and 22.8% of the tumors are from the left hemicolon and rectum respectively, 82.6% were moderately differentiated and 85.7% were invasive adenocarcinoma. Observed mutations are 74.2% in patients with advanced-stage colorectal cancer ($P = 0.006$; Table 3). The frequency of *KRAS* gene mutations observed in men (23/35; 66%) was higher than that observed in women (12/35; 34%), but the difference was not significant ($P = 0.21$) probably due to the small sample size.

Among the *KRAS* mutation-positive colorectal cancer patients 32% had isolated lung metastases compared to a significantly lower proportion of 3% in WT *KRAS* ($P < 0.005$; Table 4).

We have grouped CR, PR and SD together for the purposes of analysis. In colorectal cancer with *KRAS* mutation 68.6% had CR, PR and SD vs. 81.3% in WT *KRAS* colorectal cancer. In the mutated *KRAS* cohort 31% had disease progression compared to 19% in WT KRAS ($P = 0.182$; Table 5).

Table 2 Codon distribution of specific KRAS mutations.

Mutations	No. of patients	*P* Value*
Gly 12Asp(GGT > GAT)-----p.G12 D	16	< 0.0001
Gly 12 Val (GGT > GTT)-----p.G12 V	11	< 0.0001
Gly 12Cys (GGT > TGT) -----p.G12 C	3	< 0.0001
Gly12 Ser (GGT > AGT)-----p.G12 S	1	< 0.0001
Gly 13Asp (GGT > GAC) -----p.G13 D	4	< 0.0001

* Significant $P < 0.05$.

3.1. Multivariate analysis

The multivariate logistic regression analysis, after adjusting for baseline variables, showed that the WT colorectal cancer was associated with a 3-fold increase in its positive response to first-line chemotherapy with an odds ratio 2.83; 95% CI 0.910–8.832 (Table 6).

4. Discussion

It is widely accepted that *KRAS* mutations are among the most vital transforming genetic change occurring during colorectal

Table 4 KRAS mutation in mCRC according to metastatic site.

Metastatic sites	Mutated KRAS		WT KRAS		*P* Value
	N	%	*N*	%	
Liver	5	22.7	17	53.1	0.005
Lung	7	31.8	1	3.1	

WT: wild type; mCRC: metastatic colorectal cancer.

Table 5 KRAS mutation status and response to treatment.

Response	Mutated KRAS		WT KRAS		*P* Value
	N	%	*N*	%	
CR/PR/SD	24	68.5	39	81.2	0.182
PD	11	31.5	9	18.8	

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; WT KRAS: wild type KRAS.

Table 3 Relationship between KRAS mutation status and tumor variables.

	Mutated KRAS		WT KRAS		*P* Value
	N	%	*N*	%	
Gender					
Male	23	65.7	25	52.1	0.21
Female	12	34.3	23	47.9	
Tumor location					
Rectum	8	22.8	12	25	0.32
Sigmoid	16	45.7	18	37.5	
Ascending colon	7	20	5	10.4	
Descending colon	2	5.7	5	10.4	
Cecum	2	5.7	8	17.6	
Histologic grade					
Well differentiated	4	11.4	3	6.2	0.44
Moderately differentiated	29	82.8	39	81.3	
Poorly differentiated	2	5.7	6	12.5	
Histological type					
Invasive Adenocarcinoma	30	85.7	39	81.2	0.59
Mucinous Adenocarcinoma	5	14.2	9	18.8	
Others	–				–
Primary tumor stage					
Duke A & B	9	25.7	2	4.2	0.006
Duke C & D	26	74.2	46	95.8	
WT: wild type					

Table 6 Multivariate analysis of response to treatment in mutated KRAS colorectal cancer patients.

	Odds ratio estimates	95% CI
KRAS	2.836	0.910–8.832
Stage	0.799	0.568–1.069
Age	1.025	0.982–1.070
Histologic grade	0.466	0.119–1.820

CI: confidence interval.

tumorigenesis.[4,5] Although *KRAS* mutation is studied across the board in CRC from western countries limited data are available in mutation rates and spectrum of CRC from developing countries like Saudi Arabia. The present findings suggests a higher incidence of CRC in Saudi Arabia comparable to previous findings in Saudi Arabia[28,29] among the younger age group with more aggressive clinical manifestations compared to reports from the Western world.[23,24] The *KRAS* mutation analyses of colorectal cancer patients revealed a frequency of 42.2% which is higher than the frequency reported (~30% and ~23%) by Smith and co-workers[4] and Sameer et al.[25], respectively. This goes to emphasize the significance of geographical variation.

The liver and the lung are frequent sites of CRC metastases. Due to their respective anatomical blood vessel distribution, lung metastases are more frequent with rectal cancers while liver metastases are more common with colon cancers. However, some colon cancer patients can have lung metastases without evidence of previous liver metastases. This unascertained anatomical pattern of metastasis, observed with increasing frequency due to more precise diagnoses based on highly effectual CT scans, suggests an unusual biological mechanism of carcinogenesis in these patients.[30,31]

A recent report by Yamauchi et al.[32] of the anatomical difference between right and left colon with data from 1400 colorectal cancers from two prospective trials; the frequencies of molecular markers CIN (chromosomal instability), MSI (Microsatellite instability), CIMP (CpG island methylator phenotype and BRAF. They reported that frequencies of CIMP, MSI and BRAF mutation increased in a statistically linear fashion from the rectum to the ascending colon, while the *KRAS* mutation was high in ceacal cancer. This supports that the frequencies of molecular pathological changes in colorectal cancer evolve gradually through the bowel subsites. In our study, it looks very difficult to draw a conclusion in this regard because of the very small sample size. For example, only two patients in both ceacum and descending colon were reported as mutant *KRAS* while five and eight patients were wild type (5.7%, 10.4% and 17.6%, respectively) while the ascending colon has mutant *KRAS* in seven patients and wild type in only five patients (20% and 10.4%, respectively) again the tumor location was not of significant value ($P = 0.32$) for the *KRAS* mutation (Table 3).

It was quite consistent in our study that the prevalence of mutation is highly significant with the tumor stage 25.7% for stage Duke A and B compared with 74.2% for stage Duke C and D ($P = 0.006$).

In the present study among the *KRAS* mutation positive colorectal cancer patients the incidence of isolated lung metastases was much higher compared to WT *KRAS* irrespective of anatomical location of cancer. This finding suggests that EGFR pathway activation may allow colonic tumor cells to metastasize in the lung parenchyma avoiding an initial step of liver metastasis. This holds clinical significance in identifying patients with a greater propensity toward lung metastasis based on *KRAS* mutation. These patients need thorough workup specifically chest imaging which is not always included in the work-up for CRC. Critical analyses of lung nodules even borderline for malignancy is needed based on current findings. The fact that in present and previous[31] findings the liver was bypassed in favor of lungs supports the previous statement for more vigilant work-up inclusive of chest imaging.

Our study is supported by an elegant work of Tie et al.[33]; they reviewed three sets of patients population: A total of 148 patients with 161 resected colorectal cancer metastases were identified (cohort A), the second set was an independent clinic-based cohort of 604 patients with primary colon cancers was identified (cohort B) with stage I to IV. The third set was retrieved from 859 stage II and III patients participating in the VICTOR clinical trial (cohort C). All patients had undergone curative-intent surgery, and none had shown evidence of distant metastases at the time of surgery. They reported prevalence of 19 oncogenes among this large patient population including BRAF, KRAS, NRAS, and PIK3CA mutation.

Their data showed high concordance between the mutations in both primary and metastatic site for KRAS, BRAF, NRAS and PIK3CA.

KRAS mutation frequency was similar in liver metastases (32.3%, $P = 0.784$), but significantly higher in lung (62.0%) and brain (56.5%) metastases ($P < 0.001$ and $P = 0.004$, respectively). This confirms that KRAS mutation in the primary tumor may be associated with an increased risk of relapse in the lung or brain, but may not modify the risk of relapse in the liver.

Taken together our findings and Tie et al.[33] results support more comprehensive assessment of chest imaging prior to radical treatment and in the follow up especially during the first three years after radical surgery.

No appreciable difference could be ascertained histopathologically between *KRAS* mutated and WT CRC (P value = 0.59). On the contrary, an Indian study of 53 patients with CRC showed codon 12 *KRAS* mutation significantly associated with mucinous histotype.[25]

The *KRAS* mutation state of the tumor has been proposed as a strong marker of response to chemotherapy in mCRC.[18] With the advent of *KRAS* mutation as a negative predictive marker in colorectal cancer the EGFR monoclonal antibodies have been rapidly incorporated in our clinical practice for *KRAS* WT type colorectal cancer.[15–19] Several questions remain unanswered, large data from MRC COIN (continuous chemotherapy plus cituximab, or intermittent chemotherapy with standard continuous palliative combination chemotherapy with Oxaliplatin and a fluoropyrimidine in first-line treatment of metastatic colorectal cancer) trial showed no progression free survival advantage on addition of cituximab.[31]

A recent large study in Korean population by Kim et al.[35] also reported high prevelance of lung metastasis in patients with mutated K-ras tumors; They found mutations in 75 cases out of 143 (in both primary and metastatic sites) with 52% being mutated tumors, and lung metastasis was found in 42% of the mutated tumors while only 22% of those tumors developed liver metastasis ($P = 0.003$). Again patients with wild type tumors had more prevalence of liver metastasis than

those with mutated tumors (70% vs. 22% $P = 0.002$). More interestingly they also found higher incidence of discordance between primary tumors and metastatic site in patients who developed lung metastasis (32.4% compared with 12.3% $P = 0.005$).

The present study showed the existence of an association between *KRAS* WT and its positive response to conventional 5-FU chemotherapy irrespective of the supplementation of EGFR monoclonal antibodies.

The higher incidence of *KRAS* mutation of colorectal cancer patients in the study population is intriguing. A plausible reason is the small sample size which is not representative of the entire population. A larger study may enable a more accurate inference.

5. Conclusion

The frequency of *KRAS* mutations appears higher in the Saudi population. *KRAS* mutated colorectal cancer patients had a higher propensity for lung metastases bypassing liver metastases indicating the need for more extensive chest imaging for effective staging.

Conflict of interest

The authors declare no conflicts of interest. Funding was neither sought nor received for this study from any source.

Authors' contributions

Talha Bader contributed to data collection, interpretation and writing of the draft. Abdelsalam Ismail contributed to the study design, monitoring and conducting of the study, reviewing and discussion of the manuscript.

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;**55**:74–108.
2. Al-Eid HS, Arteh SO. Saudi cancer registry reports. Cancer incidence report Saudi Arabia 2005. 2009;18–19. <http://www.scr.org.sa>; accessed 26 May 2012.
3. Boland CR, Sinicrope FA, Brenner DE, Carethers JM. Colorectal cancer prevention and treatment. *Gastroenterology* 2000;**18**(2 Suppl. 1):S115–28.
4. Smith G, Carey FA, Beattie J, Wilkie MJ, Lightfoot TJ, Coxhead J, et al. Mutations in APC, Kirsten-ras, and p53 – alternative genetic pathways to colorectal cancer. *Proc Natl Acad Sci USA* 2002;**99**:9433–8.
5. Calistri D, Rengucci C, Seymour I, Lattuneddu A, Polifemo AM, Monti F, et al. Mutation analysis of p53, K-ras, and BRAF genes in colorectal cancer progression. *J Cell Physiol* 2005;**204**(2):484–8.
6. Ren YX, Xu GM, Li ZS, Song YG. Detection of pointmutation in K-ras oncogene at codon 12 in pancreatic diseases. *World J Gastroenterol* 2004;**10**:881–4.
7. Arends JW. Molecular interactions in the Vogelstein model of colorectalcarcinoma. *J Pathol* 2000;**190**:412–6.
8. Takayama T, Miyanishi K, Hayashi T, Sato Y, Niitsu Y. Colorectal cancer: genetics of development and metastasis. *J Gastroenterol* 2006;**41**(3):185–92.
9. Worthley DL, Whitehall VL, Spring KJ, Leggett BA. Colorectal carcinogenesis: road maps to cancer. *World J Gastroenterol* 2007;**13**(28):3784–91.
10. Kerkhoff E, Rapp UR. Cell cycle targets of Ras/Raf signalling. *Oncogene* 1998;**17**:1457–62.
11. Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenomacarcinoma sequence. *Br J Surg* 2002;**2002**(89):845–60.
12. Chong H, Vikis HG, Guan KL. Mechanisms of regulating the Raf kinase family. *Cell Signal* 2003;**2003**(15):463–9.
13. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, Zanon C, Moroni M, Veronese S, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res* 2007;**67**(6):2643–8.
14. Di Fiore F, Blanchard F, Charbonnier F, Le Pessot F, Lamy A, Galais MP, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. *Br J Cancer* 2007;**96**(8):1166–9.
15. Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison S, Wu S, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol* 2007;**25**(22):3230–7.
16. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:1626–34.
17. De Roock W, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008;**19**(3):508–15.
18. Lièvre A, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008;**26**(3):374–9.
19. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol* 2007;**25**(13):1658–64.
20. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009;**27**(5):663–71.
21. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond DF, Hayes DF, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009;**27**(12):2091–6.
22. Estrada P, Rojas-Atencio A, Zabala W, Borjas L, Soca L, Urdaneta K, et al. Frequency and clinicopathological associations of K-ras mutations in Venezuelan patients with colo-rectal cancer. *Invest Clin* 2009;**50**(1):55–63.
23. Brink M, de Goeij AF, Weijenberg MP, Roemen GM, Lentjes MM, Pachen MM, et al. K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. *Carcinogenesis* 2003;**24**(4):703–10.

24. Minoo P, Zlobec I, Peterson M, Terracciano L, Lugli A. Characterization of rectal, proximal and distal colon cancers based on clinicopathological, molecular and protein profiles. *Int J Oncol* 2010;**37**(3):707–18.
25. Sameer AS, Chowdhri NA, Abdullah S, Shah ZA, Siddiqi MA. Mutation pattern of K-ras gene in colorectal cancer patients of Kashmir: a report. *Indian J Cancer* 2009;**46**(3):219–25.
26. Weichert W, Schewe C, Lehmann A, Sers C, et al. KRAS genotyping of paraffin-embedded colorectal cancer tissue in routine diagnostics: comparison of methods and impact of histology. *J Mol Diagn* 2010;**12**(1):35–42.
27. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;**92**(3):205–16.
28. Aljebreen AM. Clinico-pathologicalpatterns of colorectalcancer in Saudi Arabia: younger with an advanced stage presentation. *Saudi J Gastroenterol* 2007;**13**(2):84–7.
29. Isbister WH. Colorectal cancer below age 40 in the Kingdom of Saudi Arabia. *Aust N Z J Surg* 1992;**62**(6):468–72.
30. Oudejans JJ, Slebos RJ, Zoetmulder FA, Mooi WJ, Rodenhuis S. Differential activation of ras genes by point mutation in human colon cancer with metastases to either lung or liver. *Int J Cancer* 1991;**49**(6):875–9.
31. Cejas P, López-Gómez M, Aguayo C, Madero R, de Castro Carpen˜o J, et al. KRAS mutations in primary colorectal cancer tumors and related metastases: a potential role in prediction of lung metastasis. *PLoS One* 2009;**4**(12), e8199, 1–6..
32. Yamauchi M, Lochhead P, Morkiawa I, Huttenhower CChen AT, et al. Colorectal cancer a tale of two sides or a continuum? *Gut* 2012;**61**(6):794–7.
33. Tie Jeanne, Lipton Lara, Desai Jayesh, Gibbs Peter, Jorissen Robert N, et al. KRAS mutation is associated with lung metastasis in patients with curatively resected colorectal cancer. *Clin Cancer Res* 2011;**17**(5):1122–30.
34. Kim Mi-Jung, Lee Hye Seung, Hyun Jee, et al. Different metastatic pattern according to the KRAS mutational status and site-specific discordance of KRAS status in patients with colorectal cancer. *BMC Cancer* 2012;**12**:347.