Annals of Oncology 16 (Supplement 4): iv44–iv49, 2005
doi:10.1093/annonc/mdi907

Prognostic and predictive factors in colorectal cancer: Kirsten Ras in CRC (RASCAL) and TP53CRC collaborative studies

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Mutations in the Ki-ras and TP53 genes are the most frequently observed genetic alterations in colorectal cancer (CRC). Ki-ras mutations are mostly found in codons 12 and 13, and less in codon 61. The majority of the TP53 mutations occur in the core domain which contains the sequence-specific DNA binding activity of the protein, and they results in loss of DNA binding.

Few centres have sufficient patients to collect detailed information in the large numbers required to determine the impact of individual ki-ras and TP53 genotypes on outcome. Moreover, it has been reported that specific genetic alterations, and not any mutation, might play a different biological role in cancer progression. For these principal reasons, two collaborative studies have been conducted (the RASCAL and the TP53-CRC Collaborative Studies) with the aim of investigating the prognostic role of any, and specific, Ki-ras and TP53 mutations in CRC progression.

The results obtained from the RASCAL studies suggest that Ki-ras mutations might have an effect on the survival rate of CRC patients, and that the specific codon 12 glycine/valine mutation might play a role in the progression of this neoplasia. The results of the TP53-CRC International Collaborative Study demonstrate the importance of primary tumor site when analyzing the prognostic value of TP53 mutations in CRC. In addition, different types of TP53 mutation might play a pivotal role in determining the biological behavior of CRC from different sites and hence the prognosis of patients. This meta-analysis produced evidence for interesting tumor site differences in the predictive value of TP53 mutation for survival benefit from 5FU chemotherapy.

Introduction

The development of colorectal cancer (CRC) is a multi-step process characterized by the accumulation of genetic alterations [1]. The Fearon and Vogelstein model assumes the involvement of the ki-ras oncogene in the transition from intermediate adenomas to carcinomas in sporadic CRC and the TP53 gene in the later events of CRC progression, when the cancer is more aggressive and is ready to produce metastases [2].

Ras is a proto-oncogene that codifies for a protein of 21 kDa (p21RAS). The Ras protein is activated transiently as a response to extracellular signals such us growth factors, cytokines, and hormones which stimulate cell surface receptors [3]. The hallmark of Ras function is a switch between an inactive state, in which the proteins are bound to guanosine diphospates (GDP) and an active state in which conversion to guanosine-triphosphates (GTP) has occurred. Approximately, 90% of the activating mutations were found in codons 12 (wild-type GGT) and 13 (wild-type GGC) of exon 1 identifying these codons as hot spot mutation points. The most frequently observed types of mutations are G → A transitions and G → T transversions. The ras gene family is made up of three different genes, known as Harvey- (H-), Kirsten- (Ki-) and N-ras. These are located in three different chromosomes and the expression levels of each one seem to be tissue specific. In particular, oncogenic mutations of Ki-ras are involved in 40% (20–50%) of CRC [4–6].

The TP53 gene is a proto-oncogene on chromosome 17. The normal TP53 protein inhibits the proliferation of cells with DNA damage. Alterations in both, or sometimes only one, of its alleles may interfere with this function. P53 point mutations were found in about 50% of colorectal cancers. The majority (∼80%) are mis-sense mutations comprising GC to AT transitions at CpG dinucleotides and occurs principally in five hotspot codons (175, 245, 248, 273 and 282) [7]. Most of these TP53 mutations occur in a region of 600 base pairs including exons 5–8, which contain the nucleotide sequences preserved during evolution and coding for the amino acids which are extremely important for the TP53 DNA binding activity (area II, codons 112–141; area III, 171–181;area IV, 234–258; and area V, 271–286) [8]. Furthermore, several functional domains, sites of mutations, have been identified within this region: the L2 loop (codons 163–195), required for the folding and stabilization of the central part of the protein; the L3 loop (codons 236–251), and the LSH motif

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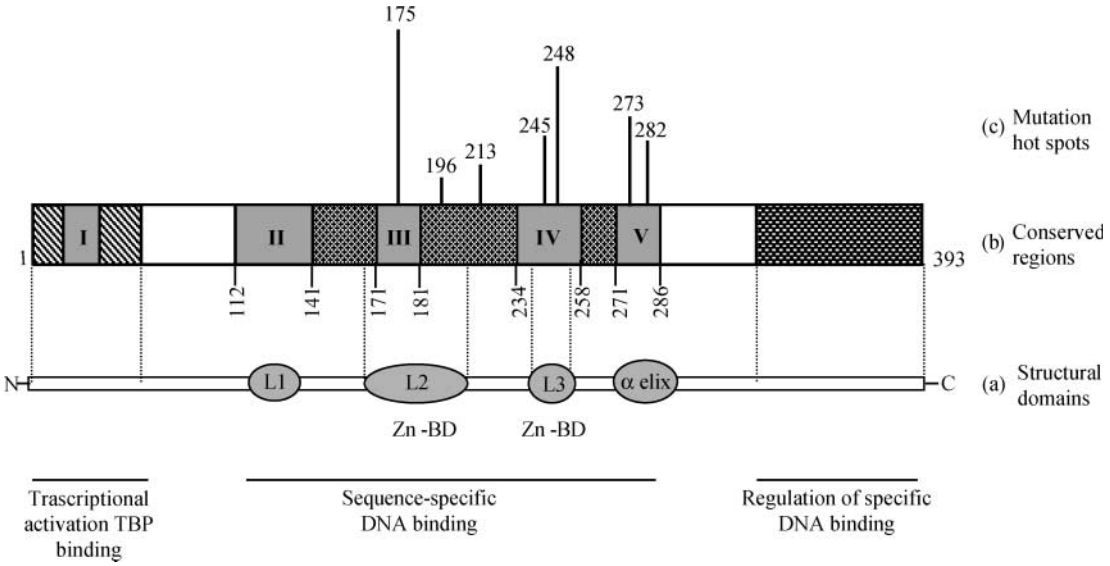

Figure 1. (a) Schematic representation of the TP53 protein structural domains, (b) highly conserved regions of p53 gene and (c) mutation hot spots in CRC as reported in literature.

(codons 273–286) within which at least two residues (241, 248 and 273, 280, respectively) contact the DNA directly [9] (Figure 1).

Most studies performed to evaluate the prognostic significance of Ki-ras and TP53 mutations in CRCs have taken into account any mutation of these genes and the results obtained thus far have proved to be rather discordant [10–16], with only some of them indicating an influence of these alterations on DFS and OS rate. Several studies have, in fact, reported a reduced survival rate in patients with Ki-ras and/or TP53 mutated tumors [10, 11], while others have found no association with prognosis [12–16]. One likely explanation of the difficulty in attributing a prognostic significance to specific Ki-ras and TP3 mutations is the insufficient statistical power of the various individual studies. Moreover, over the last few years it has been reported that specific genetic alterations, and not any mutation, might play a different biologic role in cancer progression. A study which we ourselves published, for example, reported that codon 12 mutations are associated with a mucinous phenotype while codon 13 mutations are associated with a non-mucinous phenotype which is, however, more aggressive and with greater metastatic potential [17]. With regard to TP53, several groups have reported that different types of TP53 mutation are differentially associated with CRC prognosis. These include mutations in exon 7, codon 245, conserved areas and the L3 structural domain [17–21].

Furthermore, several important issues should be considered when evaluating the prognostic significance of TP53 mutations in CRC. First, loss of TP53 function is a late event in adenoma-carcinoma progression. Second, TP53 mutations have a different incidence and perhaps also prognostic impact depending upon the site of origin of the tumor in the large bowel. The frequency of TP53 mutations is higher in distal colon and rectal tumors than in proximal colon tumors [22, 23]. Finally, several clinical studies have reported that CRC patients with wild-type TP53 derive a survival benefit from 5-fluorouracil-based chemotherapy but not those with mutant TP53 [24].

For these reasons, two collaborative studies have been conducted (the RASCAL study and the TP53-CRC Collaborative Study) with the aim of investigating the prognostic role of any, and specific, Ki-ras and TP53 mutations in CRC progression. Both of the studies had considered a large number of patients from different countries, therefore in view of the multiple statistical analyses performed, only values where $P<0.01$ were considered significant.

In particular, the RASCAL study was made up on two principal collaborative studies that have been developed in two different times. The aim of both collaborative studies was to evaluate the prognostic significance of mutations of exon 1 of Ki-ras in their entirety; the prognostic significance of specific mutations in codons 12 and 13 of Ki-ras; the association between Ki-ras mutations and the traditional clinical parameters, such as age, sex, tumor site, type of growth, Dukes' staging, grading, histological type, vascular invasion and lymphocyte response.

The aim of the *TP53-CRC International Collaborative Study* was, instead, to evaluate the prognostic and predictive significance of TP53 mutations in CRC according to site of origin in the large bowel, tumor stage, type of mutation and use of adjuvant treatment.

The RASCAL study

In the first RASCAL Study [25] the mutational status of Ki-ras gene was analyzed in 2721 patients collected from 22 centres from 13 different nations. Investigation of the mutational status of the Ki-ras gene was conducted on DNA extracted from tumor samples by various methods, for example, PCR amplification followed by SSCP analyses,

PCR and direct sequencing, PCR with allele-specific primers and hybridization. In this study, the authors show that mutations in the Ki-ras gene were important for the progression and outcome of established colorectal cancer, although some specific Ki-ras mutations (glycine/valine at codon 12) seems to have a prognostic role more important than others.

The RASCAL II study [26] analyzed data regarding 3439 cases of CRC collected from 35 centers from 19 different nations with a mean follow-up of 55 months. Five hundred and forty-eight (16%) were at Dukes' stage A, 1339 (41%) at stage B, 1056 (31%) at stage C and 436 (12%) at stage D. The same techniques of the first RASCAL Study were used to investigate the mutational status of Ki-ras gene. Thirty-five percent of the cases analyzed showed mutations in the Ki-ras gene, 26% of which in codon 12 and 9% in codon 13. About 9% of the overall mutations brought about the replacement of the amino acid glycine with valine in codon 12. The effect of the mutation was determined by two different methods—first the specific mutations were compared with other types or with wild-type, i.e. no mutations, after checking Dukes' stage, age and center of origin. Subsequently, the effect of all the mutations taken in their entirety was compared with that of the wild-type.

No association was found between Ki-ras mutations and other clinicopathological variables such as age, sex, tumor site, type of growth, Dukes' stage, histological type, vascular invasion and lymphocyte response. Multivariate analysis showed that advanced Dukes' stage, age and the codon 12 glycine/valine mutation were significantly associated with a poorer prognosis. A separate analysis of the effects of the codon 12 glycine/valine mutation in patients with stage B or stage C tumors showed that the mutation brought about a significant reduction of the disease-free interval ($P = 0.0076$, RR = 1.5) and of survival rate ($P = 0.02$, RR = 1.45) only in patients with Dukes' stage C tumors.

The TP53-CRC collaborative study

In the TP53-CRC Collaborative Study (A. Russo, V. Bazan, B. Iacopetta, D. Kerr, T. Soussi, N. Gebbia and the 'TP53-CRC' collaborative group[#], article in press) data from a total of 3,583 CRC patients from 17 different countries with information on TP53 gene mutation status were collected. Cases were divided into three groups according to the site of the primary tumor: 1017 (28%) were proximal colon, 426 (12%) were distal colon and 2031 (57%) were sigmoid colon and rectum. Follow-up times (median and range) for patients were 58 months (1–194), 61 months (1–173) and 61 months (1235) for the proximal colon, distal colon and rectal tumor groups, respectively. For the mutational analyses a total of 2397 cases were screened by PCR-SSCP followed by sequencing, 158 by PCR-DGGE followed by sequencing, 281 directly by DNA sequencing, 114 by SSCP alone and 454 by TGGE or DGGE alone. The analyses involved consideration of any TP53 mutations as well as those specific to exons 4–8 and those in regions coding for the main functional and structural domains of the protein. Mutations in the hotspot codons were also examined (codons 175, 196, 213, 245, 248, 249, 273, 282), as well as those in the denaturant codons known to have a direct effect on TP53 stability (codons 143, 175, 245, 249, 282), those in zinc binding codons (176, 179, 238, 242), those involved with DNA interaction (codons 120, 241, 248, 273, 276, 277, 280, 281, 283) and those involved in direct DNA contact (codons 248, 282) (). Analysis of point mutations (missense, nonsense), frameshift mutations (insertions, deletions) and of transitions and transversions was performed. Finally, analysis was performed of mutations that affect the following classes of amino acids: polar neutral, apolar neutral, basic and acid, together with the type of amino acid change according to the lateral group. TP53 mutations were found in 34% of the proximal colon tumors and in 45% of the distal colon and rectal tumors. TP53 mutations in the overall CRC cohort or in the three different tumor site groups did not show significant prognostic value. However the investigation of different types of TP53 mutation revealed some interesting associations, particularly for distal colon tumors. In this group, compared with tumors with wild type TP53 a poorer outcome was observed for mutations in the LSH region, denaturing mutations, multiple mutations, or mutations giving the same amino acid side group or an amino acid loss. For proximal colon tumors, only TP53 mutations in exon 5 were significantly associated with worse survival and for rectal tumors only those giving rise to an amino acid loss. In multivariate analysis adjusted for Dukes' stage, nodal status, histological grade and lymphatic invasion, only TP53 mutation associated with an amino acid loss in distal colon tumors was an independent factor for worse survival (RR = 2.52, 95% CI [1.28–4.93], $P = 0.007$). A trend toward statistical significance for poorer outcome was also observed for exon 5 mutations in proximal colon tumors (RR = 1.36, 95% CI [1.03–1.79], $P = 0.03$).

Finally, for Dukes' C patients with wild-type TP53, those treated with chemotherapy showed a significantly better survival rate in proximal colon and rectal tumor groups (Figures 2 & 3), whereas only a trend toward statistical significance ($P = 0.022$) was observed for the distal colon tumors (Figure 4). For patients with mutated TP53, a better survival rate with chemotherapy was only observed for the proximal colon tumor group. TP53 mutation had no prognostic value within patient groups treated by surgery alone or in those treated with surgery and chemotherapy.

Conclusions

The results obtained from the RASCAL studies suggest that Ki-ras mutations might have an effect on the survival rate of CRC patients, and that the specific codon 12 glycine/valine mutation might play a role in the progression of this neoplasia, thus leading to a higher risk of disease relapse or death in 30%. Furthermore, when this mutation is present in Dukes' stage C tumors, this risk goes up as far as 50%.

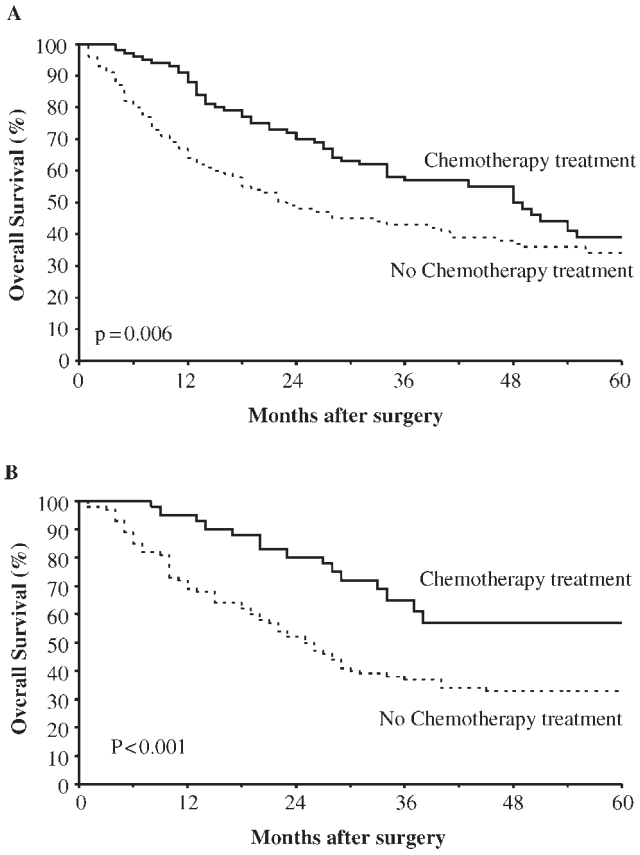

Figure 2. Dukes' C patients with both wt (A, N = 161) and mutated (B, N = 138) TP53 proximal colon cancer showed significantly improved overall survival ($P = 0.006$ and $P < 0.001$respectively) if treated with chemotherapy.

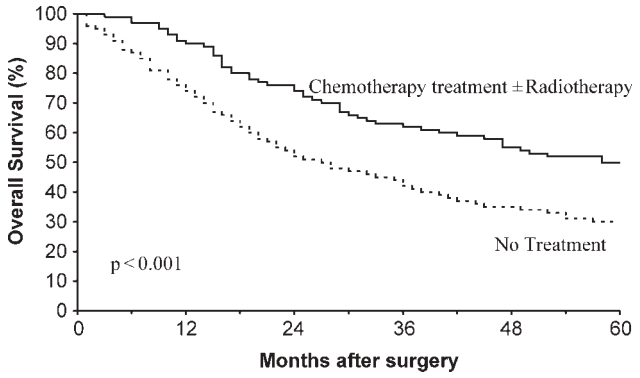

Figure 3. Only Dukes' C patients (N = 468) with wt TP53 rectal cancer showed significantly improved overall survival ($P < 0.001$) if treated with chemotherapy ∓ radiotherapy.

The results of the TP53-CRC International Collaborative Study demonstrate the importance of primary tumor site when analyzing the prognostic value of TP53 mutations in CRC. In addition, different types of TP53 mutation might play a pivotal role in determining the biological behavior of CRC from different sites and hence the prognosis of patients. This meta-analysis produced evidence for interesting tumor site differences in the predictive value of TP53 mutation for survival benefit in Dukes' C patients from 5FU chemotherapy.

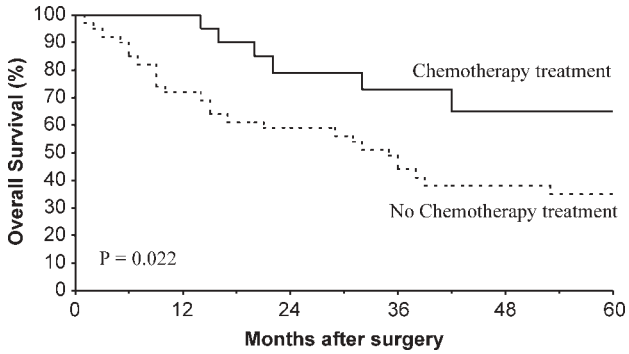

Figure 4. Only Dukes' C patients (N = 62) with wt TP53 distal colon cancer showed significantly improved overall survival ($P < 0.022$) if treated with chemotherapy.

In the light of these findings, further trials are therefore justified to evaluate the predictive significance of Ki-ras and TP53 mutations. These would require sufficient patient numbers to allow multivariate analysis and would preferably involve homogenous treatment regimens and standardized mutation screening techniques.

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Acknowledgements

We would like to thanks Mrs Pamela Gardner for help in the preparation of the text.

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