

Mutations and polymorphisms in *TP53* gene—an overview on the role in colorectal cancer

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A functionally normal TP53 is essential to protect organisms from developing cancer. Somatic mutations in the gene represent one of the highest recurring perturbations in human tumours, including colorectal cancer (CRC). However, the variegated phenotype of wide spectrum of somatic mutations in *TP53* and the complexity of the disease prevent a straight interpretation of the mutational analysis in tumours. In addition to the presence of somatic mutations, polymorphic features of the gene may also contribute to alteration of the normal *TP53* functioning and variants, mainly in the form of single nucleotide polymorphisms, can be expected to impact susceptibility to sporadic CRC. In the present study, we reviewed the potential role of alterations in the *TP53* gene, both somatic mutations and inherited sequence variations, in predisposition to CRC and in the prognosis and response to therapy. The available data from association studies have mostly shown contradictory outcomes. The majority of the studies were based on limited sample sizes and focussed on a limited number of polymorphisms, with main being the rs1042522 (Arg72Pro). Thus far, there is no possible generalisation of the role of *TP53* as also a predictor of therapeutic response and prognosis. The effects of *TP53*, and its abnormalities, on the response of tumours to cytotoxic drugs, radiation and chemoradiation are complex. However, from studies it is emerging that the inherited genetics of *TP53* pathway components could be utilised to further define patient populations in their abilities to induce p53 activity in response to either DNA damaging or p53-targeted therapies.

TP53 gene and colorectal cancer

The tumour suppressor *TP53* (MIM# 191170), located on chromosome 17p13.1, owing to diverse functions is known as ‘the guardian of the genome’ or ‘the cellular gatekeeper of growth and division’ (1,2). The gene contains 11 exons and transcribes a 2.8 kb mRNA, which is translated into a 53 kDa protein. p53, a 393 amino acid long phosphoprotein, acts as a key regulator of cellular growth control and plays a central role in the induction of genes that are important in cell cycle arrest and apoptosis following DNA damage (3). p53 undergoes several post-translational modifications that regulate its stability and its subcellular localisation (3,4).

The ability of p53 to prevent cell growth is pivotal to its tumour suppressor functions. Newly synthesised p53 accumulates in cytoplasm during G1, enters nucleus during G1–S phase transition and finally cycles back to the cytoplasm during S phase (5). The function of p53 is to reduce the risk of malignant transformation through apoptosis in cells with oncogenic activation. DNA damage and/or genotoxic stress reportedly cause p53 induction, which dependant on the context, results either in growth arrest or apoptosis (6). Cell cycle arrest, induced by p53 through transcription of downstream effectors, provides time window for the cell to repair genomic damage before entering the critical stages of DNA synthesis and mitosis. The arrested cells can be released back into the proliferating pool through various biochemical functions involving p53 (7).

TP53 is one of the most frequently mutated genes in human cancers. The most common mutations are single base substitutions that alter protein function. Some of the mutations being oncogenic confer gain-of-function properties. Germline mutations in *TP53* occur in families with Li–Fraumeni syndrome, which is associated with an increased risk of developing various cancers with an early age of onset (8). Somatic mutations at specific residues have been associated with specific clinical phenotypes in different type of cancer (9). In addition to being frequently mutated in cancers, the gene is also highly polymorphic. The fact that some germline variants in the gene may modulate the individual susceptibility to develop cancer has evinced particular interest for the potential use as predictive markers (7).

Colorectal cancer (CRC) (MIM: 114500), which is rather a common cancer, constitutes a significant proportion of the global burden of cancer morbidity and mortality (10). The worldwide incidence and mortality are estimated to be of 1.23 million new cases and 608 000 deaths in 2008 (11). Based on investigation of different stages of tumour initiation and progression, Fearon and Vogelstein proposed a model of colorectal carcinogenesis that correlated specific genetic events with evolving tissue morphology (12). Every step from normal mucosa to carcinoma involves specific and well-defined genetic alterations. This linear model has evolved to a more complex, comprehensive and mechanistic approach (13).

Characteristic alterations according to the model involve tumour suppressor genes, *APC*, *TP53* and *DCC*, and mutations in the K-ras oncogene are characteristic of that model. The mutations in *TP53* or its loss of function mainly occur at the transition from adenoma to cancer and the frequency of alterations in the gene increases with the corresponding progression of the lesion (14).

***TP53* mutations in CRC**

The reported frequency of mutations in *TP53* in CRC is ~50%, and those affect mainly five 'hotspot' codons that include 175, 245, 248, 273 and 282 (15–17). Mutation in codon 175 reportedly occurs at a high frequency in tumours located in the colon and the mutation involving the codon 288 in exon 8 is more common in rectal tumours (18). Interestingly, mutations occurring in the conserved regions of *TP53* are more frequent in tumours located at the distal compared to proximal colon and this has been suggested to reflect differences in the aetiology of CRC (19). Transversion (purine to pyrimidine) rather than transition (purine to purine, pyrimidine to pyrimidine) mutations are also reported to occur more frequently in distal colon tumours (20). Mutations in colorectal adenomas are rather rare (16% in adenomas versus 40–50% in CRC), as stated before, the alterations in the gene represent a late event in adenocarcinoma progression (21). Based on mutational data from ~9000 colorectal tumours the most frequent mutations observed in both adenocarcinomas and carcinomas have been at codons 175 and 273. However, the observed frequency of mutation at codon 248 has been reported approximately three times higher in adenocarcinomas than in adenomas (22). *TP53* mutational status in CRC appears to be connected with the age of the patients at diagnosis, but a clear explanation owing to contradictory results is lacking (23).

***TP53* variants and CRC risk**

Hitherto, 200 single nucleotide polymorphisms (SNPs) in the *TP53* gene have been identified (<http://www-p53.iarc.fr/>). Because of relatively high frequencies of the variant alleles in general population, most of the variants in *TP53* are unlikely to have more than subtle biological effects. Out of the 19 exonic polymorphisms, 8 are synonymous, which could, theoretically affect protein expression, folding and function as well as cause alternative splicing. Four out of 11 non-synonymous polymorphisms have been validated and are included in databases (HAPMAP, IARC). A large number of all variants are actually localised in introns of the gene (7).

The most investigated polymorphism is rs1042522, a G to C transversion in codon 72 of exon 4, which results in an amino acid change from arginine to proline (*TP53* Arg72Pro). The rs1042522 polymorphism is located in a proline-rich region of the protein, which has been known for long to be important for the growth suppression and apoptotic functions (24). The polymorphism results in a structural change in the protein giving rise to variants of distinct electrophoretic mobility (3). The two isoforms of p53 due to polymorphism at codon 72 differ in biochemical and biological properties. Apparently, the *TP53* Arg72 form induces apoptosis more efficiently than the Pro72 form (25,26). The Arg72 variant, when in *cis*-form with certain tumour-derived mutations, might enhance tumour suppressive function owing to increased ability to inactivate

p73. Those gain-of-function mutations are obviously influenced by the polymorphism and mutated *TP53* Arg72 bind p73 more efficiently than the mutated *TP53* Pro72 (27).

A number of studies have investigated the role of the presumptively functional Arg72Pro polymorphism in modulation of cancer risk. As indicated by several meta-analyses, Arg72Pro variant has been observed to be involved in susceptibility to breast, lung and gastric cancers but not cervical cancer (28–31). However, the epidemiological studies on association of risk with Arg72Pro polymorphism have not yielded consistent results. One of the plausible reasons for the observed inconsistency can be differential behaviour of the variant proteins in the absence or presence of mutations in the tumour. The simultaneous presence of Arg72 allele in mutated form of *TP53* (*mTP53*) may serve as a predictor of enhanced tumour development due to inactivation of p73. On the other hand, Arg72 allele over wild type (*wtTP53*) background might potentially increase apoptotic ability. A modifier effect of the Arg72Pro SNP has also been reported in germline *TP53* mutation carriers, where Arg72 was associated with an earlier age at first diagnosis of cancer (32).

Significant differences in allele frequencies for the rs1042522 polymorphism between different ethnic groups have been observed. The frequency of Pro72 allele is reported ~60% in African Americans and 30–35% in Caucasian Americans. Interestingly, the Pro72 allele frequency increases in a linear manner in multiple populations in a North to South gradient towards equator raising the possibility of a selection in areas of high ultraviolet light exposure (33). However, no significant correlation between the Arg72 allele frequency and the latitude was observed in additional 12 populations tested later in insular Southeast Asia and Oceania (34). The SNP on codon 72 has been also associated with a significant selection signal for some climate variables, such as short-wave radiation flux in winter, the associations did not remain statistical significant once correction for multiple hypothesis testing were applied in 971 unrelated individuals from 52 unique populations worldwide (35).

Several investigations have so far examined the association between the Arg72Pro polymorphism and the modulation of CRC risk including studies on risk of adenoma. The majority of the studies are based on Caucasian populations; six investigated Chinese, African and Afro-American ethnicities (Table I). Six studies have reported the association of CRC risk with Pro72 allele (36,38,39,46,47,50,54) and one with risk of adenoma (50). One study explored the association between colon cancer and 94 SNPs in 63 genes and used polymorphism interaction analysis to examine all possible combinations in 216 colon cancer patients to predict the risk. From that study it emerged that the *GSTT1* null genotype in combination with the *TP53* Pro72 allele significantly increased the risk (47). In addition to the observed statistically significant risk of cancer in the carriers of the Pro72 allele, the risk was particularly more pronounced in alcohol consumers (46). In contrast, two studies described association of the Arg72 allele with an increased risk (43,49) and one study showed increased adenoma risk (48). Interestingly, one of those studies also showed the highest frequency of Arg/Arg genotype in individuals with Dukes C and D stage of CRC (49). Similar results had been reported earlier (55). The statistically significant association was also observed for Arg72 allele homozygotes when only cancer in left colon (descending and sigmoideum) was considered (43). Meta-analyses have been recently conducted to evaluate the

Table I. Summary of association studies between CRC risk and various TP53 polymorphisms

Study	TP53 polymorphisms ^a	Cases	Controls	Ethnicity (place of the study)	Genotype analysis outcomes	Outcomes for interactions and/or haplotype analyses
Song <i>et al.</i> (36)	Arg72Pro	1829 CRC	1700	Asian (Korea)	Pro72 genotype associated with ↑ CRC risk	
Joshi <i>et al.</i> (37)	Arg72Pro	685 CRC	778	Asian (Japan)	No association	
Sameer <i>et al.</i> (38)	Arg72Pro	86 CRC	160	Asian (India)	Pro72 genotype associated with ↑ CRC risk	
Cao <i>et al.</i> (39)	Arg72Pro	156 CRC	293	Asian (Korea)	Pro72 allele associated with ↑ colon cancer risk	
Polakova <i>et al.</i> (40)	Arg72Pro PIN3 IVS7 + 72C > T Ex11 – 363G > A	614 CRC	614	Caucasian (Czech Republic)	Ex11 – 363A allele with ↓ rectal cancer risk	A ₂ -Pro72-C-G haplotype associated with ↑ CRC risk. Four other less frequent haplotypes with ↓ risk
Csejtei <i>et al.</i> (41)	Arg72Pro	102 CRC	97	Caucasian (Hungary)	No association	Pro72 allele with ↓ chance of survival
Grunhage <i>et al.</i> (42)	Arg72Pro	96 CRC	220	Caucasian (Germany)	No association	
Dakouras <i>et al.</i> (43)	Arg72Pro	93 CRC	95 Age and ethnicity matched	Caucasian (Greece)	Arg72 allele with ↑ risk of the development of CRC	Arg/Arg genotype with ↑ incidence of left colon cancer
Mammano <i>et al.</i> (44)	Arg72Pro PIN3 MspI RFLP in intron 6	90 CRC	321 Age-matched controls and 322 centenarians	Caucasian (Italy)	PIN3 A ₂ and Pro72 allele with ↓ risk of CRC	
Tan <i>et al.</i> (45)	Arg72Pro PIN3	467 CRC	563	Caucasian (Germany)	Pro72 allele with ↓ CRC risk	Protective effect of NSAIDs use for Arg72 and PIN3 A ₁ allele carriers A ₁ -Arg72 haplotype with ↓ CRC risk
Zhu <i>et al.</i> (46)	Arg72Pro C-8343G C-1863T	345 CRC	670 Sex, age, smoking and drinking-matched	Asian (China)	Arg/Pro and Pro/Pro genotypes with ↑ risk for CRC	Pro72 allele with ↑ CRC risk among the alcohol consumers
Goodman <i>et al.</i> (47)	TP53 Arg72Pro	216 Colon cancer	255	Caucasian and Afro-American (USA)	Pro72 allele with ↑ colon cancer risk	GSTT1 null polymorphism in combination with the TP53 significantly altered colon cancer risk
Koushik <i>et al.</i> (48)	Arg72Pro	856 Colorectal adenoma 442 CRC	1184 904	Caucasian (USA)	Arg72 allele with ↑ adenoma risk No association for CRC	Pro72 allele carriers at ↑ risk of proximal colon cancer in women and of distal colon cancer in men
Perez <i>et al.</i> (49)	Arg72Pro	53 CRC	109	Mixed population (mostly Argentina)	Arg72 allele with ↑ CRC risk	The prevalence of Arg72 ↑ with higher Dukes stage
Perfumo <i>et al.</i> (50)	Arg72Pro PIN3	124 Colorectal adenoma 60 CRC	188	Caucasian (Italy)	Pro72 allele with ↑ CRC and adenoma risk PIN3 A ₂ allele with ↑ adenoma risk	A ₁ -Pro72 haplotype with ↑ CRC risk A ₂ -Pro72 haplotype with ↑ adenoma risk
Kruger <i>et al.</i> (51)	Arg72Pro	126 CRC	245	Caucasian (Germany)	No association	
Buyru <i>et al.</i> (52)	G13964C	55 Colon cancer patients with HPV-positive tumours	77	Not known	No association	
Sotamaa <i>et al.</i> (53)	Arg72Pro	186 CRC (stratified for different MSI status)	323	Caucasian (Finland)	No association in any of the subgroups	

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Table 1. Continued

Study	<i>TP53</i> polymorphisms ^a	Cases	Controls	Ethnicity (place of the study)	Genotype analysis outcomes	Outcomes for interactions and/or haplotype analyses
Gemignani <i>et al.</i> (54)	Arg72Pro PIN3	374 CRC	322	Caucasian (Spain)	PIN3 A ₂ allele with ↑ risk of CRC. Weak ↑ association with CRC for Pro72 allele	
Schnieder-Stock <i>et al.</i> (55)	Arg72Pro	61 CRC 28 CRC with liver metastases	85	Caucasian (Germany)	No association	The prevalence of Arg72 ↑ with higher Dukes stage; distal tumours with ↑ LOH of the <i>TP53</i> gene more than proximal tumours
Sjalander <i>et al.</i> (56)	Arg72Pro PIN3 Intron 6	155 CRC	206	Caucasian (Sweden)	PIN3 A ₂ allele with ↓ CRC risk	A ₂ -Pro72 haplotype less frequent in CRC cases

Studies are in chronological order from the most recent to the oldest one. rs numbers for polymorphisms: Arg72Pro rs1042522, PIN3 rs17878362, IVS7 + 72C > T rs12947788, Ex11 – 363G > A rs17884306, intron 6 (or G13964C) rs17880604. For C-8343G and C-1863T, rs number was not found. LOH, loss of heterozygosity.

^aFor PIN3, the different alleles are called A₁ and A₂, with A₂ carrying the 16-bp duplication.

potential association of the codon 72 polymorphism specifically with CRC risk (57,58) or with digestive tract cancers (59) or with cancer susceptibility in general (60). In none a clear strong effect of this polymorphism on the risk of CRC emerged and these results are consistent with other studies reporting no association (37,41,42,51,53,55). One of the limitations of those studies has been the lack of information about potential gene–gene or gene–environment interactions (58).

Another frequently studied *TP53* polymorphism is 16-bp duplication in intron 3 (PIN3, rs17878362: the different alleles are called A₁ and A₂, with A₂ carrying the 16-bp duplication). A reduced amount of steady-state RNA for the variant A₂ allele in immortalised lymphoblastoid cell lines compared to the common allele was reported. These results were confirmed with mRNA extracted directly from patient's lymphocytes (54). Previously, other investigators have reported that the A₂ allele was associated with decreased apoptosis and DNA repair in lymphoblastoid cell lines (61). Consistent with these altered functional activities, several studies have correlated the intron 3 duplication with an increased risk of various cancers including lung, breast and ovary (61–65). Other groups failed to confirm those results (66,67).

Six studies examined the association between the *TP53* PIN3 polymorphism and CRC risk and one investigated the risk adenoma (Table I). While two studies showed association between the decreased risk and A₂ allele, another case-control study reported association with increased risk (44,54,56). Due to the strong linkage disequilibrium between intron 3 duplication and the codon 72 variant, it has not been discerned whether the intron 3 polymorphism alone influences mRNA stability or the effect is modulated by the presence of the Pro72 variant (54). At the same time, other investigations have failed to confirm association with increased risk in other populations with similar ethnicities (40,45,50). In an Italian population, PIN3 A₂ allele was associated with an increased risk of colorectal adenomas but not CRC (50). No significant differences were observed between cases and controls in the two by far largest studies (40,45). Interestingly, a protective effect towards CRC risk was found only among PIN3 A₁ allele carriers, who also used non-steroidal anti-inflammatory drugs (NSAIDs) (45).

Despite the large number of polymorphisms identified in the *TP53* gene, only few other variants have been analysed for association with CRC risk (Table I). No association was found with rs17880604 polymorphism in intron 6 (IVS6 – 36G > C; G13964C) (44,52,56). In one study, the carriers of the variant A-allele for the rs17884306 polymorphism in exon 11 (–363G > A) were found to be at a decreased risk of rectal cancer, while no significant association was observed with the rs12947788 (IVS7 + 72C > T) polymorphism (40).

Haplotype analysis

As evident, the contribution of individual genetic variants to the predisposition to a disease is likely to be minor and difficult to ascertain in small-sized studies with insufficient statistical power. In a modified approach, haplotype analysis has been suggested as a promising method for studying cancer-gene associations (68). It is possible that instead of individual polymorphisms, certain sets of haplotypes comprised several variants, within critical genes/loci exhibit a differential association with cancer susceptibility (69). It is assumed that these associations, and the identification of a few alleles of a haplotype block, can unambiguously identify all other polymorphic sites in the particular region, since the integration of an increasing number of common genetic variations in the analysis enables an increased statistical power in such studies. Interestingly, evidence about the relevance of specific *TP53* haplotypes on the regulation of the expression of different isoforms of the gene has emerged. An evidently cooperative effect between SNPs within the internal promoter thus emphasises the potential of inter-individual differences (70).

So far, only four studies have reported haplotype analyses based on *TP53* polymorphisms in association with CRC risk, one also investigated risk of adenoma. In two of the studies, the haplotypes included only PIN3 and Arg72Pro polymorphisms and another study also included a polymorphism in intron 6 (45,50,56). A haplotype analysis based on four polymorphisms was carried out in a large study that provided an extended coverage of the linkage disequilibrium block (40). In an earlier study, the A₁-Pro72 haplotype was weakly associated with the

risk of CRC; the A₂-Pro72 haplotype was statistically significantly less frequent in CRC patients compared to controls (56). A differential distribution of the haplotypes based on the PIN3 and Arg72Pro polymorphisms was observed between 467 CRC cases and 563 controls but only the A₁-Arg72 haplotype was associated with an increased CRC risk (45). Additionally, patients with the A₂-Pro72 haplotype and without regular use of NSAIDs were at statistically significantly decreased risk of CRC (45). A different distribution of the two most common haplotypes between cases and controls was observed in another study that included four polymorphisms of *TP53*. In particular, A₁-Arg72-C-G and A₂-Pro72-C-G were present in 81% of cases and only in 72% of controls in a total population of 614 CRC cases and 614 colonoscopy negative controls. The A₂-Pro72-C-G haplotype was also associated with an increased risk of CRC when compared with the most common haplotype with only common alleles (A₁-Arg72-C-G). Four other less represented haplotypes (A₁-Pro72-C-G, A₂-Arg72-C-G, A₁-Arg72-T-G and A₁-Arg72-C-A) were associated with significantly decreased risk. Moreover, the most common haplotype, A₁-Arg72-C-G, when compared to all other haplotypes, was associated with an increased CRC risk (40).

TP53 somatic mutations and gene variants: prognostic and predictive significance in CRC

Many studies have reported association of somatic mutations in *TP53* or abnormal protein expression with poor survival or lack of response to therapy. However, the clinical significance of *TP53* status still remains controversial (71–73). Not only the complexity of the *TP53* pathway, the abundance of mutations in different tumour types and clinical stages, and their disparate effects but also the lack of randomised prospective studies, that are compounded by heterogeneity of experimental designs, sample types and techniques make a generalised conclusion difficult. At the same time, it would perhaps be a presumptive expectation that a ubiquitously mutated gene would have the same clinical value regardless of the context (74). Structural and functional features of p53 might be useful as a molecular prognostic marker (75). It is worth taking into consideration that ‘only’ 50% of human tumours carry *TP53* mutations. Nevertheless, it is assumed that the pathway is inactivated through alterations in upstream and downstream effectors of the gene (76).

Survival predictions based on *TP53* mutational status have been observed for some malignancies. In the cancers of breast, head and neck, liver, haematopoietic and lymphoid systems, a majority of studies have associated *TP53* mutations with worsened survival. However, in the case of cancers of the bladder, brain, lung, oesophagus and ovary, data are inconsistent (18,77–82). The majority of studies on the clinical relevance of *TP53* polymorphisms mainly focused on the role of Arg72Pro. The Arg72 variant has been reported to be a more potent inhibitor of chemotherapy-induced apoptosis than the corresponding Pro72 variant (83). Patients, homozygote for Arg72 allele, with breast, lung or head and neck cancers have been shown to survive and respond better to chemotherapy and radiotherapy (84–87).

Several studies, generally with small patient numbers, have investigated the link between specific *TP53* mutations or immunohistochemical status of the protein and the prognosis and response to cancer treatment (88). In a large population-

based study, tumours with *mTP53* were shown to be associated with a significantly worse 5-year survival than those with *wtTP53*. A significantly worse prognosis was also observed for patients with specific types of mutations and mutations in proximal tumours. In multivariate analyses, however, the only significant predictors of poor prognosis were G245 hotspot mutation and mutations in proximal tumours (20).

In a review based on 18766 CRC patients from 168 studies on the effect on prognosis association of increased risk of death was observed, after correction for heterogeneity and publication bias, with both abnormal and mutated p53 (71). Another study comprised 3583 patients from 25 research groups in 17 countries and showed higher frequency of *TP53* mutations in distal and rectal than in proximal tumours; mutations were associated with lymphatic invasion in proximal tumours; amino acid loss causing mutations were associated with worse survival and patients with Dukes’ C tumours with wild type *TP53* showed better survival after treatment with adjuvant chemotherapy (89).

A few other studies have investigated the effect of *TP53* on CRC prognosis in specific subcategories including those with specific mutations, with concomitant mutations in other relevant genes, MSI status and cancer site/stage (90–94). In a study on 353 sporadic CRC patients, mutation in codon 175 of exon 5 conferred a better prognosis, while alterations in exon 8 were related with worse prognosis in different subgroups of patients that included men, patients younger than 71 years old, tumours located in the proximal colon, moderately differentiated and mucinous (18). Both *mTP53* and MSI-H resulted to be prognostic indicators for disease-free survival, but only *TP53* retained statistical significance after adjustment for clinical heterogeneity as shown in a study based on 220 tumours. Additionally, *mTP53* was associated with a short disease-free survival, whereas *KRAS* mutations did not show any prognostic significance (95).

Dukes’ B stage patients who were carriers of the variant Pro72 allele over *GSTM1* null background had poor chance of survival in a study based on 102 patients compared to patients homozygotes for Arg72 allele and *GSTM1* positive (41). The prognostic value of Arg72Pro polymorphism in colorectal adenocarcinoma may also be dependant on patient race/ethnicity. The analysis of Arg72Pro polymorphism in African American and Caucasian CRC patients showed a higher frequency of the Pro/Pro genotype in the former that associated with an increased frequency of *TP53* mutations, with advanced tumour stage and with short survival (96).

The effects of *TP53*, and its abnormalities, on the response of tumours to cytotoxic drugs, radiation and chemoradiation are complex and it is, perhaps, unrealistic to expect a straightforward relationship between any mutation in *TP53* and the response to treatment with chemotherapy (97). In particular, 5-fluorouracil (5-FU) is widely used in the treatment of a range of cancers and has shown largest impact on CRC. *TP53* can be activated by 5-FU through more than one mechanisms including incorporation of fluorouridine triphosphate into RNA, fluorodeoxyuridine triphosphate into DNA and inhibition of thymidylate synthase with resultant DNA damage (98). *TP53* status expectedly appears to have predictive value for the survival of CRC patients receiving 5-FU chemotherapy. Previous *in vitro* and clinical investigations reported that CRC patients with *wtTP53* tumours benefit from 5-FU-based chemotherapy, while those with mutated gene tumours do not (99–101). Recent studies evaluated the findings

in well-defined patient cohorts and treatment modalities [reviewed by Robles and Harris (74)].

mTP53 has been shown to be a predictor of better clinical outcome in patients with chemotherapy-refractory metastasis treated with the anti-epidermal growth factor receptor (anti-EGFR) antibody cetuximab in a study based on 64 metastatic patients. TP53 mutations are predictive markers of cetuximab sensitivity, particularly for patients without detectable KRAS mutation that confers a better response to therapy and time to progression (102). TP53 mutations and better clinical outcome may appear to be unexpected because most of studies have shown an opposite association with a worse prognosis in stage II–III CRC patients (95). However, the predictive function of TP53 mutations in metastatic CRC patients treated with targeted therapies has not been so far established. The only previous study performed on metastatic CRC in the context of targeted therapies involved anti-vascular endothelial growth factor (anti-VEGF) antibody bevacizumab that showed no correlation between TP53 status and the clinical response (103). Another study on 93 stage IV CRC patients with unresectable liver metastasis receiving exclusively 5-FU therapy did not find any association with KRAS status and TP53 alterations (104).

Thus, additionally to TP53 mutations, both in combinations and independently, the role of polymorphisms (mainly Arg72Pro polymorphism) in the gene has attracted an interest in the response to CRC therapy. *In vitro* studies using various p53-inducible isogenic cell lines showed the greater apoptotic potential of Arg72 allele both in the presence and in the absence of chemotherapeutics (105). In one study, Arg/Arg genotype of Arg72Pro polymorphism without TP53 mutations was shown to predict a more favourable response to 5-FU-based chemotherapy compared to same genotype with inactivating TP53 mutations (17). There is an indication of emerging evidence in the literature that suggests that probably inherited genetics of TP53 pathway components could also be utilised to further define patient populations in their abilities to induce p53 activity in response to either DNA damaging or p53-targeted therapies. Studies have shown that restoration of wild type p53 activity in human tumours could be employed in cancer therapy (106).

Conclusions

TP53 is a key regulator gene controlling several important cellular pathways, such as cell cycle control, apoptosis, DNA repair, and is involved in maintaining the genomic integrity. The findings that TP53 mutations can occur early in carcinogenesis, and that p53 can have a molecular signature based on the type of cancer and exposure linked to that cancer, make it an attractive target as an intermediate biomarker. In CRC, the alteration of the normal function of this gene also represents an important step in carcinogenesis although mutations are identified as late events in the process. It is assumed that TP53 mutations may help cells to cope with stress-induced tissue remodelling constraints, providing a short-term proliferative advantage, which probably leads to an enhanced risk of progression to cancer. Due to the fundamental role of TP53, naturally occurring variants are also expected to play a role in the susceptibility to sporadic CRC. Elucidation of the effect of TP53 polymorphisms on the risk of CRC is a challenge, which is attracting an interest in the recent years. No uniform conclusion can be drawn for roles of

polymorphisms and mutations in the TP53 gene as results are so far inconsistent. Further, large studies on different ethnicities investigating a wider spectrum of TP53 variants are warranted with inclusion of haplotype-tagging approach. Despite impressive progress in mechanistic understanding of p53 structure and function, p53 research has not yet generated applications of wide impact on cancer management and therapy. It is highly unlikely that p53 alterations could serve as a clinically useful, routine marker of prognosis for CRC. However, it could find clinical application for the identification of patients who might benefit from 5-FU-based chemotherapy.

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