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p53 REVIEW ARTICLE

TP53 Mutation in Colorectal Cancer

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For the p53 Special Issue

Approximately half of all colorectal cancers show p53 (TP53) gene mutations, with higher frequencies observed in distal colon and rectal tumors and lower frequencies in proximal tumors and those with the microsatellite instability or methylator phenotypes. Alterations to this gene appear to have little or no prognostic value for colorectal cancer patients treated by surgery alone, but are associated with worse survival for patients treated with chemotherapy. There is some evidence that different p53 mutations are associated with different clinical features including prognosis and response to therapy, although further large studies are required to confirm this. Several in vitro, animal and clinical studies have shown that normal p53 is required for the response of colorectal cancers to 5-fluorouracil-based chemotherapy. This should be confirmed by additional retrospective cohort studies and by the incorporation of P53 status in ongoing and future clinical trials. The evaluation of p53 overexpression, using a standardized immunohistochemical (IHC) procedure, could be a clinically useful marker for the identification of colorectal cancer patients likely to benefit from the standard chemotherapy regime currently used for this disease. Hum Mutat 21:271–276, 2003.

KEY WORDS: p53; TP53; cancer; colorectal; CRC; colon; tumor; prognosis; chemotherapy

DATABASES:
TP53 – OMIM: 191170, 151623 (LFS); GenBank: NM_000546 (mRNA)
http://p53.curie.fr/ (p53 Web Site at Institut Curie)
www.iarc.fr/P53 (IARC p53 Mutation Database)

INTRODUCTION

Since the first reports of p53 gene (TP53; MIM# 191170) mutations in colorectal cancer (CRC) more than a decade ago [Baker et al., 1989; Rodrigues et al., 1990], over 800 publications on this subject have appeared in the literature (Pubmed search, May 2002). Early work showed that growth of CRC cell lines *in vitro* could be suppressed by the introduction of wild-type p53 [Baker et al., 1990], thus establishing the tumor suppressor properties of this gene. Allelic loss of the chromosome 17p region containing the p53 gene was frequently observed in CRC [Baker et al., 1989; Delattre et al., 1989] and in conjunction with mutation of the second allele, gives rise to bi-allelic inactivation. This functional loss of p53 in CRC was proposed as a late event in the transition from adenoma to carcinoma [Baker et al., 1990; Purdie et al, 1991]. Mutation of p53 is thought to increase the protein half-life and is often associated with overexpression in the nucleus [Remvikos et al., 1990; Rodrigues et al., 1990]. The majority of translational studies carried out in the 1990s were aimed at determining whether p53 mutation and overexpression have prognostic value in CRC. For the sake of brevity, the current review will focus mostly on the results of this work and on the possible clinical implications.

METHODS USED TO DETECT TP53 ALTERATIONS

DNA sequencing has been used as the gold standard for identification of p53 mutations in cancer. Its use in primary CRC is limited however by the presence of contaminating normal DNA that can mask the detection of mutant sequence. PCR-based screening methods such as denaturing gradient gel electrophoresis (DGGE) [Hamelin et al., 1993] and single strand conformation polymorphism (SSCP) [Cripps et al., 1994] have found wider application because they do not require the tumor DNA to be pure. These methods have proven extremely useful for

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the rapid and accurate screening of large numbers of primary CRC samples for p53 mutation. Running conditions can be optimized empirically in order to attain close to 100% sensitivity [Moyret et al., 1994]. Various isotopic [Dix et al., 1994], silver stain [Bosari et al., 1995; Soong and Iacopetta, 1997], and fluorescent [Berggren et al., 2000; Makino et al., 2000; Iacopetta et al., 2000] detection systems have been developed for SSCP-based screening. Because early work found the large majority of p53 mutations to occur within conserved DNA regions located in exons 5 to 8 inclusive, most subsequent studies limited their investigation to this area.

In addition to direct molecular analysis of the p53 gene for mutation, the overexpression of p53 protein has often been used as a surrogate marker for the presence of abnormalities. The large majority of studies have used immunohistochemical (IHC) analysis, although flow cytometric methods [Remvikos et al., 1990] and a functional assay [Flaman et al., 1995] have also been reported. Different antibodies and antigen retrieval techniques have been evaluated for IHC, with the DO-7 monoclonal antibody reported as the most sensitive and specific for the detection of an underlying gene mutation [Baas et al., 1994]. The majority of workers have considered that only nuclear p53 staining indicates the presence of aberrant p53, but at least two studies have reported on the clinical significance of cytoplasmic p53 expression [Sun et al., 1992; Bosari et al., 1994]. It soon became apparent that nuclear p53 overexpression could sometimes occur in the absence of mutation and vice versa [Cripps et al., 1994; Dix et al., 1994]. Reasons proposed for the former were the accumulation of wild-type p53 in tumor cells or the failure to detect a mutation. A possible explanation for the presence of mutation in the absence of p53 overexpression is that frameshift mutations lead to truncated proteins that are not detectable by IHC. Concordance between the IHC and SSCP techniques for the detection of p53 alteration is reported to be in the range of 65–75% [Dix et al., 1994; Kressner et al., 1999; Veloso et al., 2000].

It should be emphasized that none of the p53 mutation detection techniques used in studies of primary CRC are likely to be 100% accurate. DGGE and SSCP techniques cannot be guaranteed to detect every possible single nucleotide change, even when several different running conditions are used. An ideal system for the detection of all p53 mutations in primary CRC specimens would be the use of laser capture microdissection (LCM) to purify tumor cells from frozen sections, extraction of the mRNA followed by reverse transcription, PCR amplification of the p53 cDNA, and sequencing. Unfortunately, the lack of suitably preserved tumor specimens and the relatively recent introduction of LCM technology has so far prevented such an approach. Although technically simpler than direct molecular analysis techniques and, therefore, more amenable to most routine pathology laboratories, the IHC technique gives results that are less reproducible due to the use of different fixation conditions, antigen retrieval methods, antibodies, staining protocols, and scoring systems. It is still not clear whether p53 mutation or overexpression is more strongly associated with distinctive pathological and clinical features of CRC.

FREQUENCY OF TP53 MUTATIONS AND ASSOCIATIONS WITH PATHOLOGICAL AND MOLECULAR FEATURES

In a recent overview of 14 studies that reported data on at least 50 CRC cases [Soong et al., 2000], the frequency of p53 mutation was estimated at 45% (1186/2659). Information on 1517 p53 mutations held in the UMD-p53 database [Beroud and Soussi, 2003; Soussi et al., 2000] indicates that 80% are GC to AT transitions occurring predominantly at CpG dinucleotides. These mutations are thought to arise by endogenous processes related to the deamination of 5-methylcytosine. Mutations in five hotspot codons (175, 245, 248, 273, and 282) account for approximately 43% of all p53 mutations in CRC [Soong et al., 2000; Soussi et al., 2000; Soussi and Beroud, 2003]. Three of these (codons 175, 248, and 273) contain a CpG dinucleotide. Interestingly, mutations occurring in the conserved regions of p53 are more frequent in tumors from the distal compared to proximal colon and this has been suggested to reflect a different etiology [Jernvall et al., 1997]. Transversion rather than transition mutations are also reported to occur more frequently in distal tumors, again possibly reflecting different etiology between right- and left-sided CRC [Borresen-Dale et al., 1998].

The incidence of p53 overexpression in CRC reported in the literature is generally similar to that of p53 mutation (Table 1). However, as discussed above, a number of variables associated with the IHC technique make it difficult to compare results between different studies. Both mutation and overexpression occur more frequently in distal compared to proximal tumors (Table 1) by a factor likely to be in the range of 1.5–3-fold. p53 alterations are also more frequent in tumors that are aneuploid, non-mucinous, and do not show either the microsatellite instability (MSI-) or methylator (CIMP-) molecular phenotypes. No consistent associations have been shown with other clinicopathological features including tumor stage, grade, sex or age, or with Ki-*ras* gene mutations.

PROGNOSTIC SIGNIFICANCE OF TP53 MUTATION

In the absence of a meta-analysis similar to that conducted for Ki-*ras* [Andreyev et al., 1998], this review will summarize results only from large studies

TABLE 1. Studies Examining the Association of p53 Mutation or Nuclear Overexpression With Pathological Features and Survival

Reference	*n*	IHC+ (%)	Mutation (%)	Positive associations	Prognostic significance
Starzynska et al. [1992]	107	46	nd	Higher stage	Worse survival
Yamaguchi et al. [1992]	100	61	nd	None	Worse survival
Sun et al. [1992]	293	39	nd	None	None
Bell et al. [1993]	100	45	nd	Distal tumors	None
Yamaguchi et al. [1993]	203	60	nd	Liver metastasis	Worse survival
Bosari et al. [1994]	206	46	nd	ND	Worse survival
Zeng et al. [1994]	107	47	nd	None	Worse survival
Mulder et al. [1995]	109	28	nd	Non-mucinous; metast.	None
Goh et al. [1995]	192	nd	57	ND	Worse survival[a]
Kressner et al. [1996]	294	55	nd	Aneuploid; distal	None
Smith et al. [1996]	100	25	31	ND	Worse survival[b]
Soong et al. [1997]	541	30	36	Non-mucinous; distal	Better survival[c]
Manne et al. [1997]	134	44	nd	ND	Worse survival
Poller et al. [1997]	250	61	nd	None	None
Starzynska et al. [1997]	102	46	nd	ND	None
Ahnen et al. [1998]	229	63	nd	ND	Better survival[d]
Børresen-Dale et al. [1998]	222	nd	46	Aneuploid; distal	Worse survival[e]
Tollenaar et al. [1998]	238	34	nd	ND	None
Manne et al. [1998]	504	38-63	nd	Distal tumors in whites	Worse survival[f]
Kressner et al. [1999]	191	48	52	Distal tumors	Worse survival[g]
Tortola et al. [1999]	140	nd	50	ND	Worse survival
Adrover et al. [1999]	111	42[h]	nd	None	Better survival[i]
Soong et al. [2000]	995	nd	39	Distal tumors	None
Diez et al. [2000]	190	53	nd	Distal tumors	Worse survival[j]
Samowitz et al. [2002]	1464	nd	45	Distal tumors; MSS	Worse survival[k]

[a]Worse survival associated with mutations particularly if these were within conserved regions.
[b]Worse survival associated with mutation but not overexpression.
[c]Better survival associated with overexpression in non-adjuvant treated Dukes' C and distal tumor groups.
[d]Better survival associated with overexpression in stage III; benefit from 5FU only seen in IHC-group.
[e]Worse survival associated with mutation in distal tumors and in the L3 zinc-binding domain.
[f]Worse survival associated with overexpression in proximal tumors of white, but not black, CRC patients.
[g]Worse survival associated with mutations particularly if these were outside conserved regions.
[h]Cytosolic p53 protein quantitated using luminometric immunoassay.
[i]Better survival associated with overexpression was particularly apparent for stage III group.
[j]Worse survival associated with overexpression particularly in proximal tumors.
[k]Worse survival associated with mutation in G245 hot spot and in proximal tumors.
IHC+, positive nuclear immunohistochemical staining; ND, not done; MSS, microsatellite stable.

($n \geq 100$) of p53 mutation and overexpression in primary CRC that were aimed at determining prognostic significance (Table 1). Of the 25 studies shown, 14 found an association with worse survival, 8 found no association, and 3 an association with better outcome. Several issues should be considered in the interpretation of these results. The first concerns the statistical power of the studies for detection of a survival difference between p53 mutant (p53+) and wild-type (p53−) patient groups, i.e., the prognostic value of p53 alteration (Fig. 1). Bearing in mind that about 50% of tumors are p53+ and that the 5-year survival rate from CRC is approximately 50%, studies comprising only 100 patients are statistically powered for the detection of a 30% survival difference (80% power, 5% two-sided significance level). A sample size of 800 is required in order to detect a 10% difference in survival. Clearly, the large majority of studies presented in Table 1 have insufficient statistical power to detect survival differences of less than 20–30% between p53+ and p53- patients. The second issue is the well-known publication bias against negative results although the investigations themselves may have been well executed and comprised a large sample size. This factor would tend to underestimate the number of papers in the literature that report no prognostic significance for p53 alteration.

Alteration of p53 may have different prognostic significance depending on the ethnic group [Manne et al., 1998], site of tumor origin in the colon [Sun et al., 1996; Soong et al., 1997; Manne et al., 1998; Diez et al., 2000; Samowitz et al., 2002], and stage of disease [Soong et al., 1997; Ahnen et al., 1998; Adrover et al., 1999]. Because of different antigenic specificities, the various p53 antibodies used could also identify tumor subgroups having different prognosis [Sun et al., 1992; Bosari et al., 1994]. Similarly, there is evidence that different types of p53 mutation may be associated with different prognosis [Iniesta et al., 1998; Børresen-Dale et al., 1998; Samowitz et al., 2002], although the results are sometimes contradictory [Goh et al., 1995; Kressner et al., 1999]. Another important confounding factor is the issue of adjuvant chemotherapy. There is convincing evidence that patients with wild-type p53 gain a survival benefit from the use of 5-fluorouracil (5FU)-based chemotherapy. Patients with mutant p53 (see below) do not gain this survival benefit. It is therefore critical

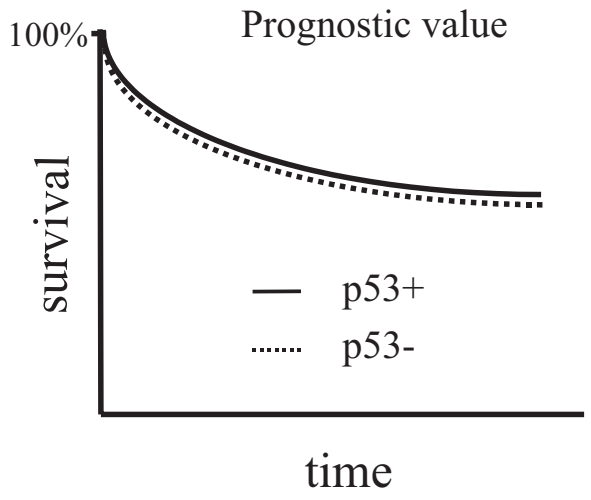

FIGURE 1. **The prognostic value of p53 alteration is determined by comparing the survival of CRC patients with a p53-altered tumor (p53+) against that of patients with wild-type p53 tumors (p53−). In the large majority of studies there is no account taken of whether or not patients received adjuvant chemotherapy.**

that the prognostic value of p53 be examined separately for patients treated with or without chemotherapy. If this is not done, then the survival rate of p53− patients relative to p53+ patients is likely to vary depending upon the proportion that received chemotherapy. The adjuvant therapy status of patients in almost all the studies shown in Table 1 was not known and may be one of the major reasons for discrepant results between different laboratories.

PREDICTIVE SIGNIFICANCE OF TP53 MUTATION

In view of the issues raised above, it is highly unlikely that p53 alteration 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 5FU-based chemotherapy. To determine the predictive value of p53, the survival rates of patients treated with or without chemotherapy are compared for both the p53− and the p53+ patient groups (Fig. 2). To date, only three studies have investigated the predictive value of p53 in CRC [Ahnen et al., 1998; Elsaleh et al., 2001; Liang et al., 2002]. Each study found that patients with normal p53 derived significant survival benefit from the use of 5FU, but not patients with mutant p53. p53 overexpression and mutation appear to provide similar predictive value [Elsaleh et al., 2001]. These clinical results are in agreement with *in vitro* and animal studies that show a requirement for normal p53 in order for colorectal tumor cells to respond to 5FU [Bunz et al., 1999].

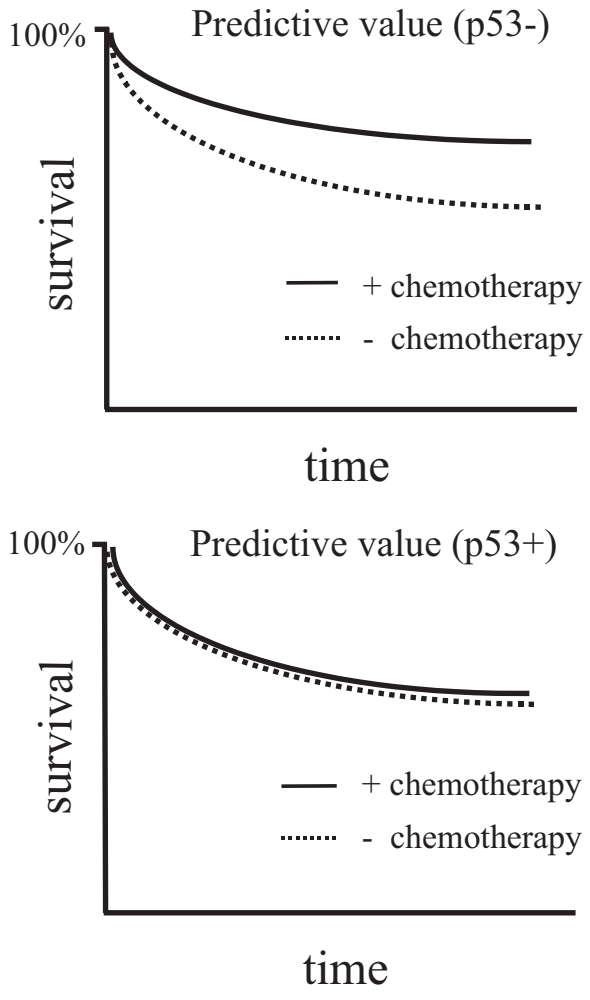

FIGURE 2. **The predictive value of p53− or p53+ is determined by comparing the survival of patients treated with or without adjuvant chemotherapy. In the three studies on CRC that have examined this to date, patients with wild-type p53 show significantly improved survival if treated with chemotherapy, but not those with mutant p53 (see text).**

SUMMARY

Mutations of p53 are found in approximately half of all CRC cases, with a higher frequency observed in distal colon and rectal tumors, and a lower frequency in proximal, mucinous, and MSI+ tumors. Alterations to this gene are likely to have very little or no prognostic significance in CRC patients treated by surgery alone, but may be associated with marginally worse survival for patients treated with chemotherapy. There is some evidence that different p53 mutations are associated with different clinical properties including prognosis and response to therapy, although further large studies are required to establish this. p53 status appears to have predictive value for the survival benefit of CRC patients from 5FU chemotherapy. This should be confirmed by additional retrospective

cohort studies and by incorporation of the p53 marker in ongoing and future clinical trials. Evaluation of p53 overexpression using a standardized IHC procedure holds considerable promise as a convenient and inexpensive means of identifying CRC patients who are likely to obtain benefit from the standard adjuvant chemotherapy regime currently in use for this disease.

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