The Impact of p53 Protein Core Domain Structural Alteration on Ovarian Cancer Survival[1]

Stephen L. Rose,[2] Andrew D. Robertson, Michael J. Goodheart, Brian J. Smith, Barry R. DeYoung, and Richard E. Buller[3]

The Holden Comprehensive Cancer Center, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology [S. L. R., M. J. G., R. E. B.], Department of Biochemistry [A. D. R.], Department of Biostatistics [B. J. S.], and Department of Pathology [B. R. D.], The University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242

ABSTRACT

Purpose: Although survival with a p53 missense mutation is highly variable, p53-null mutation is an independent adverse prognostic factor for advanced stage ovarian cancer. By evaluating ovarian cancer survival based upon a structure function analysis of the p53 protein, we tested the hypothesis that not all missense mutations are equivalent.

Experimental Design: The *p53* gene was sequenced from 267 consecutive ovarian cancers. The effect of individual missense mutations on p53 structure was analyzed using the International Agency for Research on Cancer *p53* Mutational Database, which specifies the effects of *p53* mutations on p53 core domain structure. Mutations in the p53 core domain were classified as either explained or not explained in structural or functional terms by their predicted effects on protein folding, protein-DNA contacts, or mutation in highly conserved residues. Null mutations were classified by their mechanism of origin.

Results: Mutations were sequenced from 125 tumors. Effects of 62 of the 82 missense mutations (76%) could be explained by alterations in the p53 protein. Twenty-three (28%) of the explained mutations occurred in highly conserved regions of the p53 core protein. Twenty-two nonsense point mutations and 21 frameshift null mutations were sequenced. Survival was independent of missense mutation type and mechanism of null mutation.

Conclusions: The hypothesis that not all missense mutations are equivalent is, therefore, rejected. Furthermore, p53 core domain structural alteration secondary to missense point mutation is not functionally equivalent to a p53-null mutation. The poor prognosis associated with p53-null mutation is independent of the mutation mechanism.

INTRODUCTION

Ovarian carcinoma is the deadliest gynecologic malignancy. In 2003, there will be an estimated 25,400 new cases and 14,300 deaths in the United States alone (1). The mortality rate remains high because of the delay in diagnosis that characterizes ovarian cancer. Because of this poor prognosis, many attempts have been made to identify molecular prognostic markers that may prove to be useful both diagnostically and therapeutically on an individual patient basis.

p53 is a tumor suppressor gene that encodes a M_r 53,000 nuclear phosphoprotein involved in Gap 1 (G$_1$) cell cycle arrest (2). p53 dysfunction is one of the most common genetic alterations found in cancer, occurring in up to 50% of all human malignancies (3). We and others (4–8) have shown previously that *p53* is mutated in ~60% of ovarian malignancies. In lung and ovarian cancers, *p53*-null mutations are independent molecular predictors of compromised survival (5, 9). In contrast, survival with *p53* missense mutation is highly variable. Unlike colon, lung, or breast cancer, *p53* mutation in general was not found to compromise overall survival of ovarian cancer (5, 10–12). However, p53 protein truncating or null mutations were associated with early, distant metastases (13) and, thus, not surprisingly, with compromised survival relative to individuals whose cancer harbored p53 missense mutations(5). These findings suggest a hypothesis that not all missense mutations should be expected to be functionally equivalent.

Indeed, *p53* mutations at highly conserved residues, DNA binding residues, or important residues that modify protein structure could be predicted to compromise p53 function more like a null mutation and be expected to compromise survival relative to missense mutations occurring at nonvital residues. This model, relating p53 structure to function has proven useful to prognostic outcome of colon (14), breast (15), non-small cell lung (16), as well as head and neck cancer (17).

As an alternative hypothesis, the mechanisms that give rise to *p53* mutation (slippage mismatch effecting insertion/deletion at primarily pallindromic regions, as opposed to point mutations such as seen with truncation mutations at CpG residues; Ref. 18) may be a generalized process reflective of disease aggressiveness. According to this hypothesis, one would predict that cancers rendered p53 null on the basis of point mutation might behave differently from those rendered null by frameshift mutation. To test these hypotheses and to obtain a better understanding of the impact of p53 mutation as a prognostic indicator for ovarian cancer survival, we have carried out a detailed p53 structure function analysis based upon protein modeling. In addition, we tested the hypothesis that the poor prognosis associated with p53 null mutation is independent of the origin of

mutation by stratifying the null mutations based upon point *versus* frameshift mutations.

MATERIALS AND METHODS

A total of 267 patients were seen either initially or in consultation for the diagnosis of primary, invasive epithelial ovarian cancer at The Holden Comprehensive Cancer Center of The University of Iowa. Diagnosis was between January 1, 1990, and December 31, 1998. The study was carried out in accordance with the standards of the Institutional Human Subjects Protection Review Board.

Tumor was obtained from either fresh, snap-frozen specimens ($n = 133$) or paraffin-embedded samples ($n = 134$). DNA or cDNA isolation and *p53* gene sequencing techniques have been well described in previous publications (4, 5). *p53* mutations were characterized as null (frameshift or nonsense) and missense. We did not attempt to distinguish between expressed wild-type *p53* sequence and potentially unexpressed sequences.

Because of the infrequency of *p53* gene mutations in exons 2, 3, and 11 previously reported, only exons 4–10 were studied (5, 19).[4] Exons 4–10 were directly sequenced using laser identification of fluorescent-labeled dideoxy chain-terminating nucleotides. We were unable to completely sequence some samples because of poor PCR amplification of DNA extracted from paraffin-embedded tissue. In these cases, SSCP[5] gels were performed. The more sensitive SSCP gels require smaller concentrations of clear template to identify abnormal band migration, allowing SSCP to serve as a good screening method for *p53* mutation. Forty-nine percent of all samples, both paraffin and fresh, had one or more exons examined by SSCP. In the case of abnormal SSCP gel migration pattern, DNA was re-extracted, and the exon in question was sequenced to confirm the presence or absence of mutation. Overall, we were no more likely to find a mutation from paraffin-embedded tissue than snap-frozen, indicating that artifacts were unlikely. Although some initial erroneous mutations were found in both paraffin-embedded and snap-frozen tissue, control with bi-directional sequencing and repeating individual template reactions enabled us to eliminate these false positives.

The effect of individual missense mutations on p53 structure was analyzed with the aid of the International Agency for Research p53 Mutational Database, which contains the results of a systematic automated analysis of the effects of *p53* mutations on p53 core domain structure. This analysis was developed and reported by Martin *et al.* (20) and was used with the author's permission. Mutations in the *p53* core domain were classified as either those that could be explained or not explained in structural or functional terms by their predicted effects on protein folding or on protein-DNA contacts. The source of these effects were classified as perturbations of hydrogen bonding, mutations to proline, mutations from glycine, residue clashes, mutations in DNA and zinc-binding domains, as well as those occurring in highly conserved regions of the *p53* gene. The specific codons

in question that would lead to significant structural alterations in the protein were identified based upon crystal structure examination. There were 63 residues that are 100% conserved throughout all species of *p53* sequences that were analyzed by Martin's group. These 63 residues were classified as conserved for our analysis and include: 98, 113, 120, 121, 122, 125, 127, 130, 132, 137, 139, 142, 151, 152, 158, 159, 164, 172, 173, 175, 177, 178, 179, 196, 198, 199, 205, 208, 215, 216, 218, 219, 220, 221, 223, 230, 239, 240, 241, 242, 243, 244, 245, 247, 249, 251, 253, 257, 262, 265, 266, 267, 270, 271, 272, 275, 276, 277, 278, 279, 280, 281, and 282. Mutations in DNA binding residues were defined as those in which there was at least a 5% change between the complex form of p53 observed in the crystal structure and the same structure of p53 but with the DNA removed. These were identified as Ala^{119}, Lys^{120}, Ser^{241}, Met^{243}, Asp^{247}, Arg^{248}, Cys^{275}, Ala^{276}, Cys^{277}, Arg^{280}, and Arg^{283}. Residue clashes were defined as substituted residues that resulted in three or more bad contacts with surrounding atoms in the best side chain orientation, leading to distortion of the structure and possibly incorrect protein folding. Mutations classified as those that could be explained, consisted of mutations found by analysis to significantly alter protein structure as well as those occurring in highly conserved or DNA binding codons. Mutations classified as structurally explained consisted only of the mutations found on analysis to significantly alter the protein structure. Null mutations were stratified by their mechanism of origin: point or frameshift mutations.

Statistical Analysis. Frequency tables and descriptive statistics were generated to summarize the data. The Pearson χ^2 test was used to measure the association between structural alteration and the remaining predictor variables. Kaplan-Meier plots were constructed to provide estimates of the survival functions. The multivariable effect of structural perturbation upon survival was modeled using Cox proportional hazards regression. Analyses were performed with the SPSS statistical software package (version 10.0.1; SPSS, Inc., Chicago, IL).

RESULTS

Among the 267 tumors analyzed, 82 had missense, whereas 43 contained null *p53* gene mutations. Patient follow up was last surveyed in August 2002; and at that time, median follow-up of survivors was 6.5 years. To date, 52 (63%) of 82 patients with tumor *p53* missense mutations are dead of disease. Thirty-five (81%) of 43 patients with tumor *p53*-null mutations are dead of disease. Patients who died from causes unrelated to ovarian cancer were censored appropriately. The pathological characteristics of the study cancers and their correlation with the ability to explain the effects of specific missense mutations is listed in Table 1. The effects of 62 (76%) of the missense mutations could be explained by alterations in the p53 protein, whereas 20 (24%) could not. There were no relationships between stage, grade, residual disease, or nonserous histology and explained mutations. The breakdown of the individual missense mutations and their specific structural alterations is listed in Table 2. Twenty-three (28%) of the explained mutations occurred in highly conserved regions of the p53 core protein. Kaplan-Meier survival plots were constructed based upon the structural alteration and years of follow up. There was no survival difference

---

[4] Soussi database internet address: http://www.p53.curie.fr.
[5] The abbreviation used is: SSCP, single strand conformation polymorphism.

Table 1 Tumor characteristics and percentage of explained missense mutations

	No. of patients (%)	No. explained (%)	P
Stage			0.35
I	9 (11)	7 (78)	
II	8 (10)	7 (88)	
III	53 (65)	37 (70)	
IV	12 (14)	11 (92)	
Total	82 (100)	62 (76)	
Grade			1.0
1	0	0	
2	27 (33)	20 (74)	
3	55 (67)	42 (76)	
Residual disease			0.25
≤1 cm	59 (72)	47 (80)	
>1 cm	23 (28)	15 (65)	
Histology			0.07
Serous	43 (52)	29 (67)	
Nonserous	39 (48)	33 (85)	
Exons			0.01
4	2 (2)	1 (50)	
5	24 (28)	17 (71)	
6	12 (15)	5 (42)	
7	19 (23)	16 (84)	
8	24 (29)	23 (96)	

between patients with tumor *p53* missense mutations that could be explained (median = 4.4 years) and those that were not explained (median = 3.6 years; *P* = 0.43). There was no survival difference between patients with tumor missense mutation that could only be structurally explained (median = 4.4 years) and those that could not be explained structurally (median = 4.8 years; *P* = 0.98). Similarly, there was no survival difference when missense mutations occur at highly conserved residues of the *p53* core domain (median = 4.8 years) as opposed to residues that are not highly conserved (median = 4.4 years; *P* = 0.41). There was no difference between missense mutations that occur at DNA binding residues (median = 4.0 years) *versus* those that occur at non-DNA binding regions (median = 4.8 years; *P* = 0.22). In a Cox multivariable analysis controlling for age at diagnosis, stage, grade, serous *versus* nonserous histology, and cytoreduction, explained mutations offered no prognostic significance (Table 3).

Finally, because survival of individuals with tumor *p53*-null mutations is compromised relative to those with missense mutation, we investigated survival based upon the type of *p53*-null mutation sequenced. Median survival when a mutation resulted from a point mutation (2.0 years) was identical to that when the null mutation resulted from a frameshift mechanism (1.9 years; *P* = 0.62). Overall, the presence of any null mutation significantly compromised survival relative to the presence of any missense mutation (median survival 4.0 *versus* 1.9 years; *P* = <0.01).

DISCUSSION

This is the first study to evaluate ovarian cancer survival based upon the structural alterations effected by *p53* mutation. The many sequenced *p53* mutations in ovarian cancer and the recent publication of the most systematic and complete analysis of the structural effects of *p53* point mutations (20) afforded us the opportunity to evaluate ovarian cancer survival within these parameters. Although the role of p53 null mutation as a poor prognostic factor for lung (9) and ovarian cancer has been previously established, the majority of ovarian cancers contain p53 missense mutations (5, 7).[4] Because of the highly variable course of disease when these cancers contain a missense mutation, it has been theorized that the difference may be secondary to the structural effects that an individual point mutation has upon the p53 protein. This variable hypothesis is quite plausible because it is well known that specific point mutations may cause significant changes to the three-dimensional properties of proteins, whereas others may have little, if any, effect. Support for this hypothesis is seen from the work of Reles *et al.* (21), who found decreased survival among patients with *p53* mutations in highly conserved domains of the *p53* gene, as opposed to mutations in nonconserved domains. In addition, some mutations have been shown to be flexible in that tertiary structure may vary between wild-type and mutant conformations (22). Indeed, the profiles of expression of p53 response genes can vary depending upon specific *p53* missense mutations (23).

Support for the concept that p53 structural alteration is important for cancer prognosis is obtained from the work of Webley *et al.* (24), who examined p53 in 36 primary colorectal tumors by immunoprecipitation. Their investigations characterized tumor p53 as either wild-type, mutant, or flexible using the Pab240 antibody, which recognizes an epitope displayed by mutant but not wild-type p53 (25). Apparently, cancers with flexible p53 mutation demonstrated a trend toward more aggressive tumor behavior such as distant metastases and poor cellular differentiation (24).

Taking a different approach, the impact of *p53* mutation at DNA contact residues has been studied in squamous cell carcinomas of the head and neck (17). Erber *et al.* (17) reported that *p53* mutations at these sites were associated with tumor progression and resistance to therapy when compared with structural mutations and mutations outside of the core domain. Similarly, Berns *et al.* (15) examined clinical outcomes in 66 patients whose breast cancers contained *p53* mutations. Mutations in codons directly involved with DNA binding displayed the poorest relapse-free and overall survival. We could not confirm the relevance of this observation to ovarian cancer.

Yet another critical p53 site is the L3 zinc-binding domain. Mutation at this site has been shown to predict poor survival both in patients with colorectal (14) and breast cancers (12). However, in the Borreson-Dale group study of colorectal cancer, all residues in the L3 zinc-binding domains were considered for analysis rather than focusing on the particular residues determined by crystallography to result in nonfunctional p53. Thus, overall, the adverse impact of p53 structural change is clearly supported in colon, breast, and squamous cell head and neck cancer but not in ovarian cancer.

In contrast, although we were able to show that a significant number (24%) of *p53* missense mutations in ovarian cancer could not be explained either by critical residue mutations or resultant effect upon p53 protein structure, this finding did not designate a subset of missense mutation with a better prognosis. We have not only taken into account mutations that occur in critical areas involving conserved regions, DNA and zinc binding, but also have used a systematic automated analysis of how

Table 2 Summary of tumor missense mutations and their effect upon p53 protein structure

Tumor no.	Codon	Mutation	Amino acid change	Explained	Structure explained	Reason
8.01[a]	105	GGC to GTC	Gly to Val	No		
359.01	111	CTG to CCG	Leu to Pro	Yes	Yes	Pro[b]
122.01[a]	131	AAC to CAC	Asn to His	No		
293.01[a]	132	AAG to AAT	Lys to Asn	Yes	No	Con[c]
145.01[a]	135	TGC to TAC	Cys to Tyr	Yes	Yes	Clash[d]
52.01[a]	135	TGC to TAC	Cys to Tyr	Yes	Yes	Clash
60.01	135	TGC to TAC	Cys to Tyr	Yes	Yes	Clash
1003.01	138	GCC to GTC	Ala to Val	No		
26.01[a]	138	GCC to GTC	Ala to Val	No		
234.01	151	CCC to CGC	Pro to Arg	Yes	No	Con
303.01[a]	154	GGC to GTC	Gly to Val	Yes	Yes	Gly[e]
149.01[a]	155	ACC to AAC	Thr to Asn	No		
283.01	155	ACC to AAC	Thr to Asn	No		
110.01[a]	158	CGC to CAC	Arg to His	Yes	No	Con
212.01[a]	161	GCC to ACC	Ala to Thr	No		
47.01	161	GCC to ACC	Ala to Thr	No		
683.01	171	GAG to AAG	Glu to Lys	Yes	Yes	H[f]
472.01	173	GTG to ATG	Val to Met	Yes	No	Con
2.01[a]	175	CGC to CAC	Arg to His	Yes	No	Con
210.01[a]	175	CGC to CAC	Arg to His	Yes	No	Con
308.01[a]	175	CGC to CAC	Arg to His	Yes	No	Con
512.01	175	CGC to CAC	Arg to His	Yes	No	Con
75.01[a]	175	CGC to CAC	Arg to His	Yes	No	Con
79.01[a]	175	CGC to CAC	Arg to His	Yes	No	Con
1048.01	176	TGC to TGT	Cys to Cys	No		
328.01	176	TGC to TAC	Cys to Tyr	Yes	Yes	Zn[g]
87.01[a]	179	CAT to AAT	His to Asn	Yes	Yes	Zn/con
302.01	193	CAT to CTT	His to Leu	No		
419.01[a]	193	CAT to CGT	His to Arg	No		
252.01[a]	195	ATC to ACC	Ile to Thr	No		
55.01[a]	195	ATC to ACC	Ile to Thr	No		
312.01	213	CGA to CGG	Arg to Arg	No		
270.01[a]	214	CAT to CGT	His to Arg	No		
279.01[a]	214	CAT to CGT	His to Arg	No		
155.01[a]	220	TAT to AAT	Tyr to Asn	Yes	No	Con
264.01[a]	220	TAT to TGT	Tyr to Cys	Yes	No	Con
304.01	220	TAT to TGT	Tyr to Cys	Yes	No	Con
361.01	220	TAT to TGT	Tyr to Cys	Yes	No	Con
482.01	220	TAT to TGT	Tyr to Cys	Yes	No	Con
351.01	228	GAC to TAC	Asp to Tyr	No		
663.01	236	TAC to TGC	Tyr to Cys	Yes	Yes	H
37.01[a]	237	ATG to ATA	Met to Ile	No		
17.02[a]	238	TGT to TTT	Cys to Phe	Yes	Yes	Zn/clash/h
353.01[a]	238	TGT to GGT	Cys to Gly	Yes	Yes	Zn/h
83.01[a]	241	TCC to TTC	Ser to Phe	Yes	Yes	Bind[h]/con/h
452.01[a]	244	GGC to AGC	Gly to Ser	Yes	Yes	Con/Gly
597.01	244	GGC to GTC	Gly to Val	Yes	Yes	Con/Gly
1189.01[a]	245	GGC to AGC	Gly to Ser	Yes	Yes	Con/Gly
557.01	245	GGC to GAC	Gly to Asp	Yes	Yes	Con/Gly
10.01	248	CGG to CAG	Arg to Gln	Yes	Yes	Bind
220.01[a]	248	CGG to CAG	Arg to Gly	Yes	Yes	Bind
262.01	248	CGG to CAG	Arg to Gln	Yes	Yes	Bind
477.01	248	CGG to TGG	Arg to Trp	Yes	Yes	Bind
489.01	248	CGG to TGG	Arg to Trp	Yes	Yes	Bind
535.01[a]	248	CGG to CAG	Arg to Gln	Yes	Yes	Bind
856.01	248	CGG to TGG	Arg to Trp	Yes	Yes	Bind
346.01	254	ATC to ACC	Ile to Thr	No		
116.01[a]	257	CTG to CGG	Leu to Arg	Yes	No	Con
190.01[a]	266	GGA to AGA	Gly to Arg	Yes	Yes	Con/clash
376.01	266	GGA to GAA	Gly to Glu	Yes	Yes	Con
393.01	266	GGA to GTA	Gly to Val	Yes	Yes	Con
273.01[a]	272	GTG to ATG	Val to Met	Yes	No	Con
278.01	272	GTG to TTG	Val to Phe	Yes	No	Con
103.01[a]	273	CGT to CTT	Arg to Leu	Yes	Yes	Bind/h
178.01[a]	273	CGT to CAT	Arg to His	Yes	Yes	Bind
205.01[a]	273	CGT to TGT	Arg to Cys	Yes	Yes	Bind/h

Table 2 Continued

Tumor no.	Codon	Mutation	Amino acid change	Explained	Structure explained	Reason
25.01[a]	273	CGT to GGT	Arg to Gly	Yes	Yes	Bind/h
320.01	273	CGT to CAT	Arg to His	Yes	No	Con
357.01[a]	273	CGT to CAT	Arg to His	Yes	Yes	Bind
396.01	273	CGT to TGT	Arg to Cys	Yes	Yes	Bind/h
455.01	273	CGT to TGT	Arg to Cys	Yes	Yes	Bind/h
502.01	273	CGT to CAT	Arg to His	Yes	Yes	Bind
525.01	273	CGT to CTT	Arg to Leu	Yes	Yes	Bind/h
333.01	274	GTT to TTT	Val to Phe	No		
516.01	275	TGT to TAT	Cys to Tyr	Yes	Yes	Bind/Con
401.01	278	CCT to TCT	Pro to Ser	Yes	No	Con
413.01	279	GGG to GAG	Gly to Glu	Yes	Yes	Con/clash
100.01[a]	280	AGA to GGA	Arg to Gly	Yes	Yes	Bind/h
15.01[a]	281	GAC to CAC	Asp to His	Yes	No	Con
133.1	282	CGG to TGG	Arg to Trp	Yes	No	Con
408.01[a]	282	CGG to GGG	Arg to Gly	Yes	Yes	Con/h
321.01[a]	285	GAG to AAG	Glu to Lys	Yes	Yes	H

[a] Previously reported mutations by our group.[4,26,27]
[b] Mutation to proline.
[c] Mutation in a highly conserved residue.
[d] Residue clash.
[e] Mutation from glycine.
[f] Hydrogen bond conflict.
[g] Zinc-binding domain.
[h] DNA binding residue.

Table 3 Cox multivariable analysis of individuals with explained ovarian cancer *p53* missense mutations

Variable	P
Age at diagnosis	0.72
Stage	0.02
Grade	0.59
Serous histology	0.28
Optimal cytoreduction	0.72
Explained missense mutations	0.49

these mutations will effect the three-dimensional structure of the p53 core domain. Past studies have compared all residues involved in critical areas (including p53-null mutations) to those not involved, whereas we have limited the residues to those found to predict structural alteration based upon the structural examination used by Martin's model. We have concluded from this detailed analysis that the structural alteration effected by specific missense mutations does not impact ovarian cancer survival.

This unexpected result was inconsistent with our working hypothesis. However, given the clearly adverse impact of *p53*-null mutation (median survival = 1.9 years) *versus* any *p53* missense mutation (median survival = 4.0 years), an alternate explanation must be sought as to why a mutation at, for instance, a DNA contact residue, is not functionally equivalent to a *p53*-null mutation. After all, the contact site mutation can be shown to clearly alter protein structure and, thus, should alter transcriptional events. Our alternate hypothesis was that rather than the specific mutation as the factor impacting survival, *p53* mutation may simply be a surrogate downstream event in ovarian carcinogenesis. This hypothesis was testable by looking at

clinical outcome in the cancers rendered p53 null either by point mutation (analogous to the mechanism giving rise to missense mutations) or by a frameshift error resulting from either insertion or deletion, which is thought to reflect a slippage mismatch mechanism. Unfortunately, we must reject our alternate hypothesis as well. This leaves us with the conclusion that aside from ovarian cancer rendered p53 null, we cannot explain differential clinical outcomes in the majority of advanced ovarian cancers with missense *p53* mutations found by any conventional methods of structure-function relationships, including the sophisticated Martin model.

ACKNOWLEDGMENTS

REFERENCES

1. Jemal, A., Murray, T., Samuels, A., Ghafoor, A., Ward, E., and Thun, M. J. Cancer statistics, 2003. CA - Cancer J. Clin., *53:* 5–26, 2003.

2. Israels, E. D., and Israels, L. G. The cell cycle. Oncologist, *5:* 510–513, 2000.

3. Greenblatt, M., and Harris, C. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., *54:* 4855–4878, 1994.

4. Skilling, J. S., Sood, A., Niemann, T., Lager, D., and Buller, R. E. An abundance of p53 null mutations in ovarian carcinoma. Oncogene, *13:* 117–123, 1996.

5. Shahin, M. S., Hughes, J. H., Sood, A. K., and Buller, R. E. The prognostic significance of p53 tumor suppressor gene alterations in ovarian carcinoma. Cancer (Phila.), *89:* 2006–2017, 2000.

6. Anttila, M. A., Ji, H., Juhola, M. T., Saarikoski, S. V., and Syrjanen, K. J. The prognostic significance of p53 expression quantitated by

computerized image analysis in epithelial ovarian cancer. Int. J. Gynecol. Pathol., *18:* 42–51, 1999.

7. Wen, W., Reles, A., Runnebaum, I. B., Sullivan-Halley, J., Bernstein, L., Jones, L. A., Felix, J. C., Kreienberg, R., and el-Naggar, A. p53 mutations and expression in ovarian cancers: correlation with overall survival. Int. J. Gynecol. Pathol., *18:* 29–41, 1999.

8. Baekelandt, M., Kristensen, G. B., Nesland, J. M., Trope, C. G., and Holm, R. Clinical significance of apoptosis-related factors p53, mdm2, and bcl-2 in advanced ovarian cancer. J. Clin. Oncol., *17:* 2061–2068, 1999.

9. Hashimoto, T., Tokuchi, Y., Hayashi, M., Kobayashi, Y., Nishida, K., Hayashi, S., Ishikawa, Y., Tsuchiya, S., Nakagawa, K., Hayashi, J., and Tsuchiya, E. p53 null mutations undetected by immunohistochemical staining predict a poor outcome with early-stage non-small cell lung carcinomas. Cancer Res., *59:* 5572–5577, 1999.

10. Goh, H. S., Yao, J., and Smith, D. R. p53 point mutation and survival in colorectal cancer patients. Cancer Res., *55:* 5217–5221, 1995.

11. Bergh, J., Norberg, T., Sjogren, S., Lindgren, A., and Holmberg, L. Complete sequencing of the *p53* gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. Nat. Med., *1:* 1029–1034, 1995.

12. Borreson, A. L., Anderson, T. I., Cornelis, R. S., Cornelisse, C. J., Eyfjord, J., Thorlacius, S., Borg, A., Theillet, C., Scherneck, S., Hartman, S., Hovig, E., and Devilee, P. T. p53 mutations and breast cancer prognosis: particularly poor survival rates for cases with mutations in the zinc-binding domain. Genes Chromosomes Cancer, *14:* 71–75, 1995.

13. Sood, A. K., Sorosky, J. I., Dolan, M., Anderson, B., and Buller, R. E. Distant metastases in ovarian cancer: association with p53 mutations. Clin. Cancer Res., *5:* 2485–2490, 1999.

14. Borreson-Dale, A-L., Lothe, R. A., Meling, G. I., Hainaut, P., Rognum, T. O., and Skovlund, E. T. p53 and long-term prognosis in colorectal cancer: mutations in the L3 zinc-binding domain predict poor survival. Clin. Cancer Res., *4:* 203–210, 1998.

15. Berns, E. M. J. J., van Staveren, I. L., Look, M. P., Smid, M., Klijn, J. G. M., and Foekens, J. A. Mutations in residues of TP53 that directly contact DNA predict poor outcome in human primary breast cancer. Br. J. Cancer, *77:* 1130–1136, 1998.

16. Skaug, V., Ryberg, D., Kure, E. H., Arab, M. O., Stangeland, L., Myking, A. O., and Haugen, A. p53 mutations in defined structural and functional domains are related to poor clinical outcome in non-small cell lung cancer patients. Clin. Cancer Res., *6:* 1031–1037, 2000.

17. Erber, R., Conradt, C., Homann, N., Enders, C., Finckh, M., Dietz, A., Weidauer, H., and Bosch, F. X. TP53 DNA contact mutations are selectively associated with allelic loss and have a strong clinical impact in head and neck cancer. Oncogene, *16:* 1671–1679, 1998.

18. Runnebaum, I. B., Tong, X. W., Moebus, V., Heilmann, V., Kieback, D. G., and Kreienberg, R. Multiplex PCR screening detects small p53 deletions and insertions in human ovarian cancer cell lines. Hum. Genet., *93:* 620–624, 1994.

19. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. p53 mutations in human cancers. Science (Wash. DC), *253:* 49–53, 1991.

20. Martin, A. C. R., Facchiano, A. M., Cuff, A. L., Hernandez-Boussard, T., Olivier, M., Hainaut, P., and Thornton, J. M. Integrating mutation data and structural analysis of the TP53 tumor-suppressor protein. Hum. Mutat., *19:* 149–164, 2002.

21. Reles, A., Wen, W. H., Schmider, A., Gee, C., Runnebaum, I. B., Kilian, U., Jones, L. A., El-Naggar, A., Minguillon, C., Schonborn, I., Reich, O., Kreienberg, R., Lichtenegger, W., and Press, M. F. Correlation of p53 mutations with resistance to platinum-based chemotherapy and shortened survival in ovarian cancer. Clin. Cancer Res., *7:* 2984–2997, 2001.

22. Milner, J. A conformation hypothesis for the suppressor and promoter functions of p53 in cell growth control and in cancer. Proc. R Soc. Lond., *245:* 139–145, 1991.

23. Hsiao, M., Low, J., Dorn, E., Ku, D., Pattengale, P., Yeargin, J., and Haas, M. Gain-of-function mutations of the *p53* gene induce lympho-hematopoietic metastatic potential and tissue invasiveness. Am. J. Pathol., *145:* 702–714, 1994.

24. Webley, K. M., Shorthouse, A. J., and Royds, J. A. Effect of mutation and conformation on the function of p53 in colorectal cancer. J. Pathol., *191:* 361–367, 2000.

25. Legros, Y., Meyer, A., Ory, K., and Soussi, T. Mutations in p53 produce a common conformational effect that can be detected with a panel of monoclonal antibodies directed toward the central part of the p53 protein. Oncogene, *9:* 3689–3694, 1994.

26. Buller, R. E., Skilling, J. S., Sood, A. K., Plaxe, S., Baergen, R. N., and Lager, D. J. Field cancerization: Why late "recurrent" ovarian cancer is not recurrent. Am J Obstet Gynecol., *178:* 641–649, 1998.

27. Buller, R. E., Lallas, T. A., Shahin, M. S., Sood, A. K., Hatterman-Zogg, M., Anderson, B., Sorosky, J. I., and Kirby, P. A. The p53 mutational spectrum associated with BRCA1 mutant ovarian cancer. Clinical Cancer Research, *7:* 831–838, 2001.

Clinical Cancer Research

The Impact of p53 Protein Core Domain Structural Alteration on Ovarian Cancer Survival

Stephen L. Rose, Andrew D. Robertson, Michael J. Goodheart, et al.

Updated version	Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/9/11/4139

Cited articles	This article cites 26 articles, 11 of which you can access for free at: http://clincancerres.aacrjournals.org/content/9/11/4139.full#ref-list-1
Citing articles	This article has been cited by 3 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/9/11/4139.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/9/11/4139. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.