Distant Metastases in Ovarian Cancer: Association with *p53* Mutations[1]

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ABSTRACT

Distant metastases are unusual occurrences at presentation and during the progression of epithelial ovarian cancer. There are no good clinical predictors of this phenomenon. Because *p53* dysfunction is common in ovarian cancer, we chose to investigate whether specific types of mutations predicted a predisposition to distant metastasis. We hypothesized that the complete absence of intact p53 protein as seen with *p53* null mutations may be associated with an enhanced tendency to develop distant metastatic disease. The complete coding sequence of 130 tumor DNA samples was screened for *p53* mutations by single-strand conformational polymorphism analysis. Abnormal single-strand conformational polymorphism findings were correlated with the specific DNA sequence abnormalities and outcome. Ninety-four (72%) tumors carried *p53* mutations. Sixty-two were missense mutations, and 32 were null mutations (6 nonsense mutations, 23 frameshift mutations, and 3 splice-site mutations). Twenty-eight patients were found to have distant metastases (pericardium, brain, parenchymal liver, spleen, or lung) either at presentation or during the course of their treatment. Distant metastases were nearly 8-fold more common in patients whose tumors carried a null mutation (66%) than in those with either missense mutations (8%) or wild-type p53 (8%; $P < 0.001$). When a null mutation was present, 25% of the tumors were associated with distant metastases at initial diagnosis. No individual with wild-type *p53* or a missense mutation in the tumor presented with distant metastasis. Tumors with null mutations were more likely to be associated with lymph node metastasis ($P = 0.003$), advanced stage (stage III/IV; $P < 0.001$) and high grade (grade II/III; $P < 0.001$), at presentation. These tumors progressed with distant metastases more swiftly than did tumors with either missense mutations (mean, 1.18 *versus* 2.71 years; $P = 0.04$) or wild-type *p53* (3.57 years; $P = 0.015$). In contrast to the popular dogma, distant metastases in ovarian cancer do not necessarily result from prolonged treatment of disease. They may be predicted to occur early in the disease course due to a specific molecular genetic abnormality: null mutation of the *p53* tumor suppressor gene. These findings need to be carefully considered when choosing between regional *versus* systemic treatment modalities.

INTRODUCTION

Ovarian cancer usually presents with widespread intra-abdominal metastasis. However, the disease remains confined to the peritoneal cavity at presentation and throughout its course approximately 85% of the time (1). Occasionally, patients present with aggressive disease, manifested by parenchymal liver or lung metastases, or develop metastases to such distant sites as the brain during disease progression (2). To predict the course of disease and clinical outcome, investigators have developed strategies using clinical parameters and biomarkers like CA125 (3, 4). However, these traditional parameters are often of limited use for selecting biologically different subpopulations. The ability to predict clinical outcome accurately would be of benefit to both the patient and her physicians. More importantly, therapy could be tailored based upon such predictive factors.

To metastasize, a tumor cell must overcome a number of factors. These include entrance into the vascular system, travel to a distant site while avoiding immune surveillance, localization in the microvasculature of the future metastatic site, growth, and evolution of a blood supply (5, 6). At the molecular level, studies have begun to characterize a growing number of factors that control these steps. Representative factors include the matrix metalloproteinases, tissue inhibitors of metalloproteinase, cathepsins, E-cadherin, NM23, thrombospondin, and VEGF[3] (7–12). Expression of thrombospondin (an inhibitor of angiogenesis) and VEGF is modified by the *p53* tumor suppressor gene (7, 8, 13, 14). Whereas p53 overexpression correlates with poor clinical outcome for many cancers (15–19), only a few studies have evaluated the role of p53 dysfunction in the development of tumor metastasis (19–22). Only two of these studies have related actual *p53* mutations rather than protein overexpression to metastasis. Despite the suggestion by Kupryjanczyk *et al.* (23) that p53 protein accumulation accelerates the metastatic spread of the primary tumor, we (24) and others (25, 26) have not been able to relate immunostaining or *p53* mutations to

Received 2/2/99; revised 6/1/99; accepted 6/28/99.

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[1] Supported in part by American Cancer Society Institutional Seed Grant IRG-IN-122N (to R. E. B.) and by the American College of Obstetricians and Gynecologists-Ortho academic training fellowship (to A. K. S.).

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[3] The abbreviations used are: VEGF, vascular endothelial growth factor; SSCP, single-strand conformational polymorphism.

the stage of ovarian cancer at diagnosis. Recently, we have found that *p53* null mutations carry a significantly worse prognosis than *p53* missense mutations.[4] To further understand the role of p53 dysfunction in metastasis, we have carried out a complete molecular analysis of *p53* mutations in a large cohort of ovarian cancer patients. We hypothesize that *p53* null mutations resulting in the complete absence of p53 protein are associated with a higher likelihood of developing distant metastases.

MATERIALS AND METHODS

Patients. A total of 199 patients with ovarian cancer underwent surgery at the University of Iowa between December 1990 and December 1996. Tumor samples were available from 130 of 199 (65%) patients. Approval was obtained from the Institutional Review Board for collection and molecular analysis of tumor samples.

Diagnosis and classification of all tumors were verified by pathology review at our institutional gynecological oncology tumor board. The cancer was staged in accordance with the International Federation of Gynecology and Obstetrics surgical staging system. Primary papillary peritoneal and fallopian tube carcinomas were included in the study group because of their biological and clinical similarity to ovarian cancer. Low malignant potential tumors were not included in this study. The traditional follow-up for patients with ovarian malignancies after completion of initial treatment includes physical examination and CA125 determination every 2 months for the first year, every 3 months for the second through third years, and every 6 months for the fourth and fifth years. A baseline computed tomography scan is usually obtained at the completion of initial therapy including chemotherapy. A chest radiograph is obtained annually. Long-term follow-up was available for all patients.

Distant metastasis was defined as parenchymal disease (liver and spleen) or extra-abdominal disease other than in the lymph nodes. Pleural effusion in the absence of parenchymal lung disease was not counted as distant metastasis because effusions can also occur due to a transudative process across the diaphragm. Therefore, the International Federation of Gynecology and Obstetrics surgical staging system was not used to define distant metastasis. In addition, staging systems are static and do not take into account the development of distant metastasis in the future.

Preparation of Tissue and DNA Isolation. Tumor samples were snap-frozen at the time of surgery in liquid nitrogen. DNA isolation and preparation techniques have been reported previously (24). Paraffin-embedded samples were prepared by the sonication technique (27).

Detection of *p53* Mutations. Ovarian cancers were screened for mutations in the entire coding sequence (exons 2–11) of the *p53* gene using PCR and SSCP analysis as we have reported previously (24). Tumor DNA with suspicious migratory patterns on SSCP analysis was sequenced using intron-based γ-^{32}P-end-labeled primers and the fmol DNA sequencing system (Promega Biotech), as described previously (24). Both strands of the DNA product from the PCR were sequenced to check for fidelity. Abnormalities were verified by resequencing the same region, using products from a separate (independent) PCR to avoid mistaking an early-cycle PCR error as a mutation.

p53 allelic loss was assessed as described previously by the intron 1 *(Alu)* repeat, codon 72 polymorphism, and the absence of normal coding sequence when a specific *p53* mutation was identified (28, 29).

Statistical Analysis. The χ^2 test was used to determine whether a relationship existed between variables using Stat Graphic software (Statistical Graphics Corp., Rockville, MD). The Cox proportional hazards model was used to perform stepwise multiple linear regression for the selection of significant predictors of distant metastases. $P < 0.05$ was considered statistically significant.

Table 1 Relationship between distant metastases and clinicopathological variables in ovarian cancer

Variable	Distant metastases: No (n = 101)	Distant metastases: Yes (n = 29)	P
Age (yrs)	58.9 (31–84)[a]	59.9 (38–89)[a]	0.70
Histology			
Serous	66	22	0.29
Other	35	7	
Stage			
I/II	26	1	0.009
III/IV	75	28	
Grade			
I	14	0	0.03
II/III	87	29	
Ascites			
Yes	72	28	0.004
No	29	1	
Cytoreduction			
Optimal	68	14	0.06
Suboptimal	33	15	
Lymph nodes			
Positive	12	8	0.001
Negative	32	1	
Not done	58	19	

[a] Age range.

RESULTS

Tumors from 130 patients were evaluated in this study. The average age of patients at the time of diagnosis was 59.1 years (range, 31–89 years). Papillary serous tumor was the most common histological type (68%). Other histological types included mucinous (6%), endometrioid (21%), clear cell (3%), malignant mixed Mullerian (1%), and transitional cell (1%) tumors. A total of 103 of 130 (79%) patients presented with advanced-stage disease (stage III or IV) at the time of diagnosis. In addition, most patients (89%) presented with high-grade (grade II or III) disease.

Twenty-nine patients (22%) were found to have distant metastases either at initial presentation or during the course of their treatment. Clinicopathological variables for patients with and without distant metastases were evaluated and are reported

[4] M. S. Shahin, A. K. Sood, J. I. Sorosky, B. Anderson, J. H. Hughes, and R. E. Buller. Prognostic significance of *p53* tumor suppressor gene alterations in ovarian cancer, manuscript in preparation.

Table 2 Spectrum of *p53* mutations

p53 mutation	No. (%)
Insertion/Deletion	23 (17)
Nonsense	6 (5)
Missense	62 (48)
Splice	3 (2)
None	36 (28)

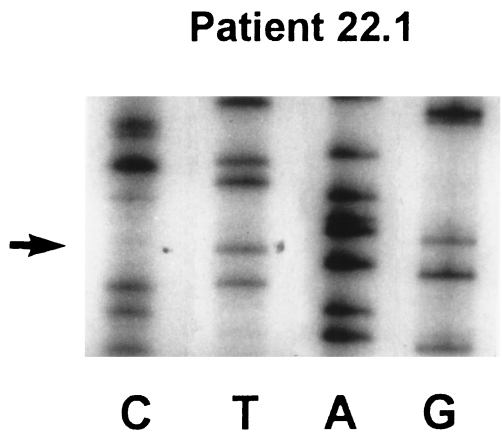

Fig. 1 Sequence analysis of the *p53* gene, exon 5, showing a *C*AG→*T*AG mutation in patient 22.1 resulting in a null mutation.

in Table 1. There were no significant differences between the two groups with regard to age, serous histology, and the likelihood of optimal surgical cytoreduction. Patients who developed distant metastases were more likely to have high-grade tumors ($P = 0.03$) and present with advanced-stage disease ($P = 0.009$). They were also more likely to have ascites at the time of presentation ($P = 0.004$). Among patients who had lymph node sampling performed, patients who developed distant metastases were more likely to have lymph node metastases ($P = 0.001$).

p53 mutation analysis was performed on all patients for exons 2–11, and the results are shown in Table 2. SSCP analysis revealed migratory shifts suggestive of mutations in 95 tumors. Sequence analysis revealed mutations in 94 (72%) tumors. Of all mutations, 23 (25%) were frameshift mutations, and 62 (66%) were missense mutations. There were six (6%) nonsense mutations and three (3%) splice mutations. The frameshift, nonsense, and splice mutations comprise the null mutations. Fig. 1 shows a sequence analysis from patient 22.1, who was found to have a nonsense mutation in exon 5 that caused the wild-type sequence CAG (glutamine) to be changed to TAG (stop). The clinical course of disease in this patient was unusual. She underwent optimal surgical cytoreduction for stage IIIB papillary serous ovarian adenocarcinoma followed by six cycles of paclitaxel and cisplatin. A reassessment laparotomy after completion of chemotherapy was negative. Within 14 months, the patient developed cerebellar metastasis that was resected, followed by whole brain radiation therapy. The patient died of disease 9 months later.

Clinicopathological variables were also analyzed with regard to *p53* mutation status, which was classified as no mutation, missense mutation, or null mutation (Table 3). There were no significant differences in age, tumor histology, and the ability to optimally cytoreduce tumor burden at the time of initial cytoreductive surgery between the three groups. Tumors with null mutations were more likely to present with malignant ascites ($P = 0.02$) and advanced-stage disease ($P < 0.001$) when compared to those with missense mutations or wild-type *p53*. Similarly, those with null mutations were also more likely to present with high-grade disease ($P < 0.001$). Fifty-three patients underwent lymph node sampling during their initial surgery. Tumors with null mutations were more likely to have metastatic spread to the lymph nodes (75%) than those with missense mutations (37%) or wild-type *p53* (12%; $P = 0.003$).

Table 4 shows the correlation of *p53* mutations with development of distant metastases in patients with ovarian cancer. Five of 62 patients (8%) with missense *p53* mutations and 3 of 36 patients (8%) with wild-type *p53* developed distant metastases. In contrast, 66% of tumors with null mutations developed distant metastases ($P < 0.001$). Among the 21 tumors with null mutations and distant metastases, 90% were associated with complete absence of the other wild-type allele.

Table 3 Relationship between *p53* mutations and clinicopathological variables in ovarian cancer

	p53 mutation			
Variable	None ($n = 36$)	Missense ($n = 62$)	Null ($n = 32$)	*P*
Age (yrs)	57.3 (31–84)[a]	59.3 (37–89)[a]	60.0 (44–82)[a]	NS[b]
Histology				
Serous	22	40	26	0.16
Other	14	22	6	
Stage				
I/II	15	10	2	<0.001
III/IV	21	52	30	
Grade				
I	10	4	0	<0.001
II/III	26	58	32	
Ascites				
Yes	24	46	30	0.02
No	12	16	2	
Cytoreduction				
Optimal	25	41	16	0.20
Suboptimal	11	21	16	
Lymph nodes				
Positive	2	9	9	0.003
Negative	15	15	3	
Not done	19	38	20	

[a] Age range.
[b] NS, not significant.

Table 4 Distribution of ovarian cancer metastases by *p53* mutation type

	p53 Mutation			
Variable	None ($n = 36$)	Missense ($n = 62$)	Null ($n = 32$)	*P*
Distant metastases				
No	33	57	11	<0.001
Yes	3	5	21	

Table 5 Spectrum of *p53* mutations and distant metastases in ovarian cancer

Fam. no.	Mutation type	Exon	Mutation[a]	Specifics[b]	Site of metastasis	Time[c] (yrs)
198	Deletion	5	13111 del 32→ter 169	TGCAGCTGTGGGTTGATTCCACACCCC-CGCCCGGCACCCG[d]	Liver	0
96.11	Deletion	4	12108 del 4→ter 121	ATGAAGCT	Liver	0
314	Deletion	4	12108 del 4→ter 121	ATGAAGCT	Liver	0
213	Insertion	6	13362 ins T→ter 208	AATTTTGCGT[d]	Liver	0
350	Deletion	6	13393 del T→ter 246	ACACTTTTC	Liver and lungs	0
131.2	Deletion	5	13207 del C→ter 246	GCTGCCCCC[d]	Liver	0
					Lungs	1.02
420	Deletion	8	14546 del G→ter 344	GAAAGGGGA	Liver	0
31	Deletion	4	12139 del G→ter 122	CCCCGCGTG[d]	Mediastinum	0
316	Insertion	4	12209 ins 17→ter 128	ATCTCCTGGCCCCTGTCATCTTCTG	Liver	0.33
317	Deletion	9	14751 del TC→ter 335	CTTCAG	Liver	0.42
100	Deletion	4	12108 del 4→ter 121	ATGAAGCT	Liver	0.67
88	Deletion	5	13231 del TA→ter 207	CAGATAGCGA[d]	Liver	1.13
278	Missense	8	Val 272 Phe	CTG→TTG	Liver	1.38
61	Deletion	6	13331 del C→ter 246	CCCTCCTCA[d]	Liver	1.42
60	Missense	5	Cys 135 Tyr	TGC→TAC	Liver	1.52
81	Deletion	7	14010 del AC→ter 238	TCTGACTGTA[d]	Liver	1.52
22.1	Nonsense	5	Gln 165 Ter	CAG→TAG	Brain	1.58
3	Deletion	7	14049 del C→ter 246	GTTCCTGC[d]	Brain	1.83
29.2	None				Liver	1.82
159	Deletion	8	14571 del C→ter 322	CCCCCAGGG[d]	Liver	1.83
311	Deletion	8	14496 del 56→ter 304	TGTGCCTGTCCTGGGAGAGACCGGCGCACAGA-GGAAGAGAATCTCCGCAAGAAAGGGGAGCCTC	Liver and spleen	1.91
103	Missense	8	Arg 273 Leu	CGT→CTT	Liver	2.17
8	Missense	4	Gly 105 Val	GGC→GTC	Liver	3.72
20	Deletion	7	14075 del 7→ter 323	GAGGCCCATCCTCAC[d]	Liver	3.98
107.2	None				Liver	4.25
288	None				Lung and pericardium	4.62
76	Splice	9		CAGgt→CAGac	Liver and lungs	3.75
11	Missense	5	Val 172 Phe	GTT→TTT[d]	Liver	4.75
84	Deletion	8	14566 del C→ter 344	AGCTGCCCC[d]	Brain	4.75

[a] For deletion and insertion mutations, the mutation description contains the nucleotide, the deletion (del) or insertion (ins), the specific nucleotides changed (if <3) or the number of base pairs changed (if >2), and the codon in which the frameshift results in a termination (ter) signal. For nonsense and missense mutations, the wild-type amino acid and affected codon are described.

[b] Mutation sites are underlined.

[c] Time to development of distant metastasis. Time 0 reflects the presence of distant metastasis at the time of presentation.

[d] Previously reported mutations (24, 35).

The specific *p53* mutations associated with distant ovarian cancer metastases are listed in Table 5, along with the sites where the metastases were detected. Parenchymal liver involvement was the most common site of distant metastasis, occurring in 24 of 29 patients (83%) with distant metastases. For 20 patients (69%), the liver was the sole site of distant metastasis. Brain (three patients) and mediastinal (one patient) metastases were also encountered. Three patients developed metastases to the liver and lungs, one developed metastases to the liver and spleen, and one developed metastases to the lungs and pericardium.

Eight of 29 patients (28%) with distant metastases presented with extraperitoneal metastases at the time of initial diagnosis, whereas 21 developed distant metastases during the course of their treatment. A total of 72% of all distant metastases developed within 2 years of initial diagnosis. Eight of 32 patients (25%) with null mutations initially presented with distant metastases. Tumors with null mutations were more likely to develop distant metastases more quickly (mean, 1.18 years) than those with missense mutations (mean, 2.71 years; $P = 0.04$) or wild-type *p53* (mean, 3.57 years; $P = 0.015$).

All patients with distant metastases diagnosed at the time of initial presentation had *p53* frameshift mutations. Nineteen of 23 patients (83%) with frameshift mutations developed distant metastases. In contrast, 1 of 3 patients (33%) with a splice site mutation and 1 of 6 patients (17%) with a nonsense mutation developed distant metastases ($P = 0.004$).

Significant univariate variables (*p53* null mutation, high stage, high grade, nodal metastasis, and presence of ascites) were considered as possible independent predictors of distant metastasis using the Cox proportional hazards model. *p53* null mutation was the most significant predictor of distant metastasis ($P < 0.001$), followed by stage ($P = 0.002$). The other factors were not significant in the multivariate analysis.

DISCUSSION

Prognostic factors in patients with cancer are sought to learn about the natural progression of disease and to predict outcome for individual patients. Additionally, these factors may help identify patients for whom the failure of conventional treatment can be predicted in advance. Such patients become

candidates for novel gene-specific therapeutic strategies based upon specific molecular determinants of treatment failure.

Ovarian cancer is the second most common gynecological malignancy in the United States. Most patients with ovarian cancer present with stage III or higher disease and require adjuvant treatment. However, even in patients with advanced-stage disease, the cancer is usually confined to the peritoneal cavity (1). A number of authors have evaluated traditional clinical parameters such as histology, grade, stage, and ascites as predictors for poor outcome in patients with ovarian cancer (3, 30, 31). Unfortunately, the course of ovarian cancer is highly variable, and the standard clinical predictors of distant metastases and poor outcome have met with limited success. Thus, novel approaches to understand and predict the course of disease are needed.

Development of distant metastases in the liver, brain, and other sites is uncommon (2, 32). The traditional explanations for the development of distant metastases in ovarian cancer patients have included exposure to multiple chemotherapy agents and disruption of the blood-brain barrier by chemotherapeutic agents (2). However, if these mechanisms were the most significant factors, why are distant metastases so uncommon? Whereas chemotherapy may play a role in a fraction of patients who develop distant metastases, we propose that there are specific molecular mechanisms operative in most of these tumors that explain their aggressive behavior.

The loss of tumor suppressor function of the p53 protein subsequent to a mutation in the coding sequence seems to be a feature common to many cancers, including ovarian cancer. Wild-type *p53* gene product has been shown to play a role in many cellular functions including cell cycle regulation and apoptotic cell death (33). *p53* expression has been evaluated as a predictor of the course of disease in various cancers, including ovarian cancer. Kim *et al.* (34) evaluated 101 gastric tumors and found a significant correlation between *p53* overexpression, lymph node metastasis, and distant metastasis. Similarly, Silvestrini *et al.* (18) demonstrated that *p53* overexpression provided significant prognostic information in breast cancer patients and was an independent predictor of distant metastasis. Studies of ovarian cancer outcome based upon immunohistochemical staining have yielded conflicting results (16, 25). However, specific *p53* mutations have not been evaluated with regard to the development of distant metastases. We hypothesized that patients with p53 protein-truncating alterations (null mutations) would be at the greatest risk for developing distant metastases, based upon their demonstrated poor survival. Consistent with this hypothesis, 66% of tumors with null mutations developed distant metastases compared to only 8% of tumors with missense mutations and 8% of tumors with wild-type *p53* ($P < 0.001$). Tumors with null mutations also developed their distant metastases more quickly (mean, 1.18 years) than tumors with missense mutations or no mutations ($P = 0.015$). Even after incorporating the traditional clinical prognostic factors, multivariate analysis showed that *p53* null mutation was the most significant predictor of distant metastasis.

Although specific *p53* mutations have not been correlated with clinical behavior in ovarian cancer, there are other tumor models suggesting aggressive tumor behavior with null mutations. Ruttledge *et al.* (35) demonstrated that among patients with neurofibromatosis, individuals with protein-truncating mutations (null mutations) in the *NF2* gene on chromosome 22 were significantly more likely to exhibit severe disease compared to those with missense alterations ($P < 0.001$). Tomlinson *et al.* (36) have reported a 16-bp deletion in exon 7 of the *p53* gene in an aggressive medulloblastoma that developed an incisional site metastasis 6 months after complete resection and radiation therapy. Thus, the findings presented in the current study are consistent with other tumor models.

It is possible that the correlation between *p53* null mutations and aggressive tumor behavior manifested by distant metastases may be downstream of more fundamental molecular abnormalities. We have previously shown that genomic instability, which is common in ovarian cancer (37), strongly correlates with the development of *p53* frameshift mutations (38). Thus, for some ovarian cancers, genomic instability is the cause of the *p53* mutations rather than the result of *p53* dysfunction. In the present study, patients with frameshift mutations developed distant metastases more frequently than patients with other types of mutation ($P = 0.004$). Genomic instability has been shown to correlate with distant metastases in prostate cancer (39) and in melanoma (40). Whether genomic instability is the fundamental event that gives rise to distant ovarian cancer metastases remains to be demonstrated.

Vascularization of the tumor is another mechanism that may directly contribute to the development of distant metastasis in ovarian cancer. Microvessel density and p53 protein expression correlate with the metastasis of head and neck squamous cancers (19). Both lymphatic spread and hematogenous spread of colon cancers have been shown to be related to the presence of *p53* mutations (22). At the molecular level, vascular penetration appears to be related to the up-regulation of VEGF and the down-regulation of thrombospondin, both of which are, at least in part, controlled by the *p53* gene product (7, 8). It is tempting to speculate that localized intra-abdominal spread of ovarian cancer may be influenced by *p53* missense mutations, whereas extra-abdominal spread is due to a complete failure of null mutations to suppress VEGF and a complete loss of thrombospondin.

Our findings have important clinical implications. Given the high likelihood of developing distant metastases in patients with null mutations, these patients may not be appropriate candidates for localized treatments such as i.p. chemotherapy, ^{32}P, or targeted gene replacement. Therefore, we would encourage physicians to consider *p53* mutational analysis on all newly diagnosed patients with ovarian cancer. When null mutations are encountered, consideration should be given to novel treatment approaches including systemic *p53* gene replacement therapy (41, 42).

REFERENCES

1. Vaccarello, L., Rubin, S. C., Vlamis, V., Wong, G., Jones, W. B., Lewis, J. L., and Hoskins, W. J. Cytoreductive surgery in ovarian carcinoma patients with a documented previously complete surgical response. Gynecol. Oncol., *57:* 61–65, 1995.

2. Geisler, J. P., and Geisler, H. E. Brain metastases in epithelial ovarian carcinoma. Gynecol. Oncol., *57:* 246–249, 1995.

3. Ansell, S. M., Rapoport, B. L., Falkson, G., Raats, J. I., and Moeken, C. M. Survival determinants in patients with advanced ovarian cancer. Gynecol. Oncol., *50:* 215–220, 1993.

4. Buller, R. E., Vasilev, S., and DiSaia, P. J. CA125 kinetics: a cost effective clinical tool to evaluate clinical trial outcomes in the 1990s. Am. J. Obstet. Gynecol., *174:* 1241–1254, 1996.

5. Kohn, E. C., and Liotta, L. A. Molecular insights into cancer invasion: strategies for prevention and intervention. Cancer Res., *55:* 1856–1562, 1995.

6. MacDonald, N. J., and Steeg, P. S. Molecular basis of tumour metastasis. Cancer Surv., *16:* 175–199, 1993.

7. Volpert, O. V., Dameron, K. M., and Bouck, N. Sequential development of an angiogenic phenotype by human fibroblasts progressing to tumorigenicity. Oncogene, *14:* 1495–1502, 1997.

8. Kieser, A., Weich, H. A., Brandner, G., Marme, D., and Kolch, W. Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene, *9:* 963–969, 1994.

9. Tamakoshi, K., Kikkawa, F., Nawa, A., Ishikawa, H., Mizuno, K., Tamakoshi, A., Yamagata, S., Suganuma, N., and Tomoda, Y. Characterization of extracellular matrix-degrading proteinase and its inhibitor in gynecologic cancer tissues with clinically different metastatic form. Cancer (Phila.), *76:* 2565–2571, 1995.

10. Mandai, M., Konishi, I., Komatsu, T., Mori, T., Arao, S., Nomura, H., Kanda, Y., Hiai, H., and Fukumoto, M. Mutation of the *nm23* gene, loss of heterozygosity at the *nm23* locus and k-*ras* mutation in ovarian carcinoma: correlation with tumour progression and *nm23* gene expression. Br. J. Cancer, *72:* 691–695, 1995.

11. Scambia, G., Panici, P. B., Ferrandina, G., Salerno, G., D'Agostino, G., Distefano, M., diVincenzo, R., Ercoli, A., and Mancuso, S. Clinical significance of cathepsin D in primary ovarian cancer. Eur. J. Cancer, *30A:* 935–940, 1994.

12. Darai, E., Scoazec, J. Y., Walker-Combrouze, F., Mlika-Cabanne, N., Feldmann, G., and Madelenat, P. Expression of cadherins in benign, borderline, and malignant ovarian epithelial tumors: a clinicopathologic study of 60 cases. Hum. Pathol., *28:* 922–928, 1997.

13. Mukhopadhyay, D., Tsiokas, L., and Sukhatme, V. P. Wild-type *p53* and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. Cancer Res., *55:* 6161–6165, 1995.

14. Dameron, K. M., Volpert, O. V., Tainsky, M. A., and Bouck, N. Control of angiogenesis in fibroblasts by *p53* regulation of thrombospondin-1. Science (Washington DC), *265:* 1582–1584, 1994.

15. Buttitta, F., Marchetti, A., Gadducci, A., Pellegrini, S., Morganti, M., Carnicelli, V., Cosio, S., Gagetti, O., Genazzani, A. R., and Bevilacqua, G. p53 alterations are predictive of chemoresistance and aggressiveness in ovarian carcinomas: a molecular and immunohistochemical study. Br. J. Cancer, *75:* 230–235, 1997.

16. Levesque, M. A., Katsaros, D., Yu, H., Zola, P., Sismondi, P., Giardina, G., and Diamandis, E. P. Mutant p53 protein overexpression is associated with poor outcome in patients with well or moderately differentiated ovarian carcinoma. Cancer (Phila.), *75:* 1327–1338, 1995.

17. Victorzon, M., Nordling, S., Haglund, C., Lundin, J., and Roberts, P. J. Expression of p53 protein as a prognostic factor in patients with gastric cancer. Eur. J. Cancer, *32A:* 215–220, 1996.

18. Silvestrini, R., Benini, E., Veneroni, S., Daidone, M. G., Tomasic, G., Squicciarini, P., and Salvadori, B. p53 and *bcl-2* expression correlates with clinical outcome in a series of node-positive breast cancer patients. J. Clin. Oncol., *14:* 1604–1610, 1996.

19. Gasparini, G., Weidner, N., Maluta, S., Pozza, F., Boracchi, P., Mezzetti, M., Testolin, A., and Bevilacqua, P. Intratumoral microvessel density and p53 protein: correlation with metastasis in head-and-neck squamous-cell carcinoma. Int. J. Cancer, *55:* 739–744, 1993.

20. Fontanini, G., Vignati, S., Bigini, D., Mussi, A., Lucchi, M., Angeletti, C. A., Basolo, F., and Bevilacqua, S. Bcl-2 protein: a prognostic factor inversely correlated to p53 in non-small-cell lung cancer. Br. J. Cancer, *71:* 1003–1007, 1995.

21. Faille, A., De Cremoux, P., Extra, J. M., Linares, G., Espie, M., Bourstyn, E., DeRocquancourt, A., Giacchetti, S., Marty, M., and Calvo, F. *p53* mutations and overexpression in locally advanced breast cancers. Br. J. Cancer, *69:* 1145–1150, 1994.

22. Goh, H. S., Chan, C. S., Khine, K., and Smith, D. R. *p53* and behaviour of colorectal cancer. Lancet, *344:* 233–234, 1994.

23. Kupryjanczyk, J., Thor, A. D., Beauchamp, R., Merritt, V., Edgerton, S. M., Bell, D. A., and Yandell, D. W. *p53* gene mutations and protein accumulation in human ovarian cancer. Proc. Natl. Acad. Sci. USA, *90:* 4961–4965, 1993.

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

25. Klemi, P. J., Pylkkanen, L., Kiilholma, P., Kurvinen, K., and Joensuu, H. p53 protein detected by immunohistochemistry as a prognostic factor in patients with epithelial ovarian carcinoma. Cancer (Phila.), *76:* 1201–1208, 1995.

26. Marks, J. R., Davidoff, A. M., Kerns, B. J., Humphrey, P. A., Pence, J. C., Dodge, R. K., Clarke-Pearson, D. L., Iglehart, J. D., Bast, R. C., Jr., and Berchuck, A. Overexpression and mutation of *p53* in epithelial ovarian cancer. Cancer Res., *51:* 2979–2984, 1991.

27. Heller, M. J., Burgart, L. J., TenEyck, C. J., Anderson, M. E., Greiner, T. C., and Robinson, R. A. An efficient method for the extraction of DNA from formalin-fixed, paraffin-embedded tissue by sonication. BioTechniques, *1:* 372–377, 1991.

28. Skilling, J. S., Powills, K., Lager, D. J., Anderson, B., Sorosky, J., and Buller, R. E. *p53* allelotypes and enhanced detection of allelic loss in ovarian cancer: lack of correlation with familial and clinical factors. Gynecol. Oncol., *61:* 180–188, 1996.

29. Buller, R. E., Sood, A., Fullenkamp, C., Sorosky, J., Powills, K., and Anderson, B. The influence of the *p53* codon 72 polymorphism on ovarian carcinogenesis and prognosis. Cancer Gene Ther., *4:* 239–245, 1997.

30. Marsoni, S., Torri, V., and Valsecchi, M. G. Prognostic factors in advanced epithelial ovarian cancer. Br. J. Cancer, *62:* 444–450, 1990.

31. Omura, G. A., Brady, J. F., Homesley, H. D., Yordan, E., Major, F. J., Buchsbaum, H. J., and Park, R. C. Long-term follow-up and prognostic factor analysis in advanced ovarian carcinoma; the Gynecologic Oncology Group experience. J. Clin. Oncol., *9:* 1138–1150, 1991.

32. Seewaldt, V. L., Figge, D. C., Greer, B. E., Tamimi, H. K., Brown, W. S., and Cain, J. M. Primary central nervous system recurrence after paclitaxel therapy for epithelial ovarian malignancy. Gynecol. Oncol., *55:* 456–458, 1994.

33. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res., *51:* 6304–6311, 1991.

34. Kim, K. M., Lee, A., Chae, H. S., and Shim, S. I. Expression of p53 and NDP-K/nm23 in gastric carcinomas: association with metastasis and clinicopathologic parameters. J. Kor. Med. Sci., *6:* 406–413, 1995.

35. Ruttledge, M. H., Andermann, A. A., Phelan, C. M., Claudio, J. O., Han, F. Y., Chretien, N., Rangaratnam, S., MacCollin, M., Short, P., Parry, D., Michels, V., Riccardi, V. M., Weksberg, R., Kitamura, K., Bradburn, J. M., Hall, B. D., Propping, P., and Rouleau, G. A. Type of mutation in the neurofibromatosis type 2 gene (*NF2*) frequently determines severity of disease. Am. J. Hum. Genet., *59:* 331–342, 1996.

36. Tomlinson, F. H., Jenkins, R. B., Scheithauer, B. W., Keelan, P. A., Ritland, S., Parisi, J. E., Cunningham, J., and Olsen, K. D. Aggressive medulloblastoma with high-level N-myc amplification. Mayo Clin. Proc., *69:* 359–365, 1994.

37. Sood, A. K., and Buller, R. E. Genomic instability in ovarian cancer: a reassessment using an arbitrarily primed polymerase chain reaction. Oncogene, *13:* 2499–2504, 1996.

38. Sood, A. K., Skilling, J. S., and Buller, R. E. Ovarian cancer genomic instability correlates with *p53* frameshift mutations. Cancer Res., *57:* 1047–1049, 1997.

39. Egawa, S., Uchida, T., Suyama, K., Wang, C., Ohori, M., Irie, S., Iwamura, M., and Koshiba, K. Genomic instability of microsatellite repeats in prostate cancer: relationship to clinicopathological variables. Cancer Res., *55:* 2418–2421, 1995.

40. Usmani, B. A., and Sherbet, G. V. Homologous recombination in variants of the B16 murine melanoma with reference to their metastatic potential. J. Cell. Biochem., *61:* 1–8, 1996.

41. Hurford, R. K., Dranoff, G., Mulligan, R. C., and Tepper, R. I. Gene therapy of metastatic cancer by *in vivo* retroviral gene targeting. Nat. Genet., *10:* 430–435, 1995.

42. Lesoon-Wood, L. A., Kim, W. H., Kleinman, H. K., Weintraub, B. D., and Mixson, A. J. Systemic gene therapy with *p53* reduces growth and metastases of a malignant human breast cancer in nude mice. Hum. Gene Ther., *6:* 395–405, 1995.

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Clin Cancer Res 1999;5:2485-2490.