

# A Molecular Genetic and Statistical Approach for the Diagnosis of Dual-Site Cancers

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**Background:** Concurrent tumors can be synchronous, independently derived, non-metastatic tumors or metastatic tumors. The prognosis and clinical management of patients with these different concurrent tumor types are different. **Methods:** DNA from normal and tumor tissues of 62 patients with synchronous endometrial and ovarian, bilateral ovarian, or endometrial and bilateral ovarian tumors was analyzed for loss of heterozygosity and microsatellite instability using eight polymorphic microsatellite markers at loci frequently deleted in ovarian and/or endometrial cancers. A statistical algorithm was designed to assess the clonal relationship between the tumors. **Results:** The original histopathology reports classified 26 (42%) case patients with single primary tumors and related metastatic lesions and 21 (34%) with independent primary tumors; 15 (24%) were unclassified. Genetic data identified 35 (56%) case patients with single primary tumors and related metastatic lesions, 18 (29%) with independent primary tumors, and nine (15%) that could not be typed. Excluding case patients with histopathology reports for which a clonal relationship was uncertain or was not reported, there was 53% concordance between genetic and histopathology diagnoses. Increasing the stringency of the statistical analysis increased the number of uncertain diagnoses but did not affect the proportion of discordant genetic and histologic diagnoses. **Conclusions:** We have developed a rapid and robust combined genetic and statistical method to establish whether multiple tumors from the same patient represent distinct primary tumors or whether they are clonally related and therefore metastatic. For the majority of case patients, histopathology reports and genetic analyses were in agreement and diagnostic confidence was improved. Importantly, in approximately one-fourth of all case patients, genetic and histopathologic analyses suggested alternative diagnoses. The results suggest that genetic analysis has implications for clinical management and can be performed rapidly as a diagnostic test with paraffin-embedded tissues. [J Natl Cancer Inst 2004;96:1441–6]

Patients with concurrent tumors are a well-recognized and frequently encountered problem in clinical oncology. In such case patients, it is important to establish whether the tumors have arisen independently of each other or whether one tumor has arisen as a metastasis of the other (1–7). Patients diagnosed with multiple, independently derived, primary tumors versus those diagnosed with a single primary tumor with related metastatic lesions may have a different prognosis and/or response to therapy and will therefore have different treatment options.

Currently, distinguishing between a primary tumor with related metastatic lesions and independent primary tumors in-

volves clinicopathologic interpretation based on multiple criteria including histologic type and grade (8–11). In general, if tumors at different sites have dissimilar histologic features they are generally regarded as independently derived primary tumors (12). Alternatively, the two tumors may have a similar histologic appearance but could still represent independent tumors (13,14). One way to avoid uncertainty in distinguishing the relationship between different tumors would be to develop a diagnostic method that relies on the genetic characterization of tumors that could then be used to complement existing histopathology approaches to diagnosis. Previous studies suggest that genetic markers, such as the pattern of X-chromosome inactivation and microsatellite analysis, can accurately predict whether different tissues from the same patient share a common clonal origin (15–19). Furthermore, recent advances in technology indicate that such analyses can be performed rapidly and reliably (20).

To assess the ability of genetic analysis to accurately diagnose tumors with independent origins, we compared two diagnostically challenging scenarios in gynecologic oncology. One scenario is the occurrence of synchronous tumors of the ovary and endometrium (2,4,12,21–23). This is not an infrequent occurrence—many patients with endometrioid cancer of the ovary have a concomitant endometrial tumor, and ovarian involvement of an endometrial cancer occurs in 5%–8% of patients at laparotomy (24–26). A patient with a primary ovarian adenocarcinoma and an endometrial metastasis would represent FIGO (The International Federation of Gynecology and Obstetrics) stage 2A ovarian cancer, and a patient with a primary endometrial adenocarcinoma and an ovarian metastasis would represent stage 3 endometrial cancer (assuming no other sites were involved). Two separate primary tumors confined to the ovary and uterus would represent two stage 1 cancers; such a patient would have a better prognosis than a patient who has metastatic disease and, depending on the substage of the tumors, might not require

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adjuvant treatment involving chemotherapy or radiotherapy (2,22,27). The other scenario addressed in this study is the occurrence of bilateral ovarian tumors. Frequently, patients with ovarian cancer have bilateral ovarian disease. Occasionally both ovaries will be involved without breach of the ovarian capsule or extra-ovarian spread of the cancer (FIGO Stage 1B) (28). These bilateral ovarian tumors may have arisen independently and simultaneously. Alternatively, one tumor may have arisen as a metastasis of the other, via a mechanism that is not yet known (29,30).

The aims of the current study were to 1) develop a combined genetic and statistical method to establish the clonal relationship between concurrent tumors arising in the same patient that is quantifiable, reliable, and robust, 2) use this method to establish whether a series of synchronous ovarian/endometrial and bilateral ovarian tumors represent either independently derived primary tumors or a single primary tumor with metastatic lesions, and 3) compare genetic diagnoses of these cancers with the original histopathology diagnosis.

## PATIENTS AND METHODS

### Patient Samples

Sixty-two patients who had been diagnosed with synchronous endometrial and epithelial ovarian cancer, bilateral ovarian cancer, or bilateral ovarian and endometrial cancers were identified from the pathology database at St. Bartholomew's Hospital and through collaboration with the Royal Marsden Hospital, London; Addenbrooke's Hospital, Cambridge; and the Royal Hospitals Trust, Belfast. For each patient, we obtained the original histopathology reports that provided information about histologic subtype, tumor grade, and the pathologist's opinion as to whether the multiple synchronous tumors represented independently derived primary tumors or primary tumors with related metastatic lesions.

Also included in the study were samples from seven patients of synchronous tumors that were known from clinical diagnoses and/or histology reports to represent either metastases of each other or independent primary tumors. Tumors from these seven case patients were used to validate genetic analyses. In six of these patients—four with tumors occurring in regional lymph nodes and omentum, one in which the tumor occurred in a regional lymph node of a primary breast cancer, and one in which an ovarian tumor was a confirmed metastasis of a gastrointestinal cancer—tumors from the same patient were considered metastases. In one patient with ovarian cancer, an incidental neurofibroma was considered an independent primary event with respect to the ovarian cancer.

Ethics committee approval was obtained for all patient tissues used in this study from the East London and City Health Authority Tissue Subcommittee (No. T01002). Written consent for the use of stored tissue blocks was obtained from all patients.

### Genetic Analysis

DNA from normal and tumor tissues was extracted following microdissection of archival, paraffin-embedded tissue sections. Tumor samples were taken from regions of tissue containing more than 80% neoplastic cells. Normal samples were taken either from non-neoplastic areas adjacent to tumor tissue or from non-neoplastic tissue sections. Microdissection was performed

either manually using a glass pipette or 0.2- $\mu$ m-bore needle to isolate distinct regions of tissue or, when the areas of tissue were less clearly defined, using a PixCell Laser Capture Microdissector (Arcturus, Braintree, U.K.). DNA extraction was performed by incubating tissue samples in 500  $\mu$ L of lysis solution (100 mM Tris-HCl [pH 8], 2 mM EDTA, and 400  $\mu$ g/mL Proteinase K [Boehringer Mannheim, Lewes, U.K.]) at 56 °C for 5 days as previously described (31). The extracted DNA was purified by phenol-chloroform extraction and ethanol precipitation. DNA samples were resuspended in 100  $\mu$ L of ddH<sub>2</sub>O and were analysed using the polymerase chain reaction (PCR); routinely, 5  $\mu$ L of DNA sample was used in a 15- $\mu$ L PCR.

DNA samples were analysed for loss of heterozygosity (LOH) and microsatellite instability (MSI) using a panel of eight polymorphic microsatellite markers at five chromosomal loci that had previously been shown to be frequently deleted in ovarian and/or endometrial cancers (Table 1). Markers on the same chromosome arm were grouped together. Markers on chromosome arms 17p and 17q were considered to undergo LOH independently ( $P = .79$ , Fisher's exact test). Primer sequences are available upon request. PCR primers were labeled (Sigma-Genosys, Haverhill, U.K.) with fluorescent FAM, HEX, or TAMRA dyes, and amplified products were separated using a 3700 capillary DNA sequencer (Applied Biosystems, Warrington, U.K.). Data were analysed using Genotyper software (Applied Biosystems). LOH was interpreted as complete or partial reduction in intensity of an allele in the tumor sample compared with the normal sample. In the case of partial reduction, the ratio of the intensity of allele 2 (a2) to that of allele 1 (a1) was compared between normal DNA and tumor DNA. Partial reduction was defined as a ratio of normal a2/a1 to tumor a2/a1 of less than 0.5 or greater than 1.5. MSI was interpreted as the addition of one or more alleles, different in size than the allele(s) observed in the normal sample. In all instances in which LOH or MSI were detected, the analysis was repeated to confirm the results.

### Statistical Analysis

A statistical algorithm was designed to assess the clonal relationship between different tumors from the same patient based on the fingerprint of genetic alterations. The analysis examined the probability that one tumor arose as a clone of the other or that the two tumors arose independently of each other based on similarities or differences in the pattern of genetic changes between the two neoplasms. For example, if two tumors

**Table 1.** Microsatellite markers used for genetic fingerprinting in this study and the frequencies of loss of heterozygosity (LOH) described in endometrial and ovarian cancers from previously published data (32–41).

Microsatellite marker	Chromosome location	% LOH frequency by tumor type	
		Ovary	Endometrium
D17S520	17p12	0.57	0.21
TP53	17p13.1		
D17S1329	17q21	0.55	0.09
CACNLB1	17q21–17q22		
D11S902	11p15–11p13	0.42	0.11
D11S922	11p15.5		
D22S156	22q11.2	0.47	0.12
D10S187	10q24–10q25	0.14	0.47

from the same patient showed loss at the same microsatellite locus, but the allele that was lost in each case was different, then this would suggest that the tumors were clonally unrelated. However, if two tumors showed loss of the same allele at one or more microsatellite loci, this would suggest that the tumors shared a common clonal origin. Microsatellite markers showing MSI were excluded from the statistical algorithm.

The statistical approach adopted was based on the model shown below. At each of the chromosomal regions examined, five mutually exclusive outcomes may be observed. The likelihoods ( $L$ ) for the different observable outcomes are:

$$L = \begin{cases} 1 - \alpha & \text{Not informative} \\ \alpha(1 - \lambda_c)(1 - \lambda_o)(1 - \lambda_e) & \text{Informative, no allele loss detected} \\ \alpha(1 - \lambda_c)\lambda_o(1 - \lambda_e) & \text{LOH detected in the ovarian} \\ & \text{but not in the endometrial tumor} \\ \alpha(1 - \lambda_c)(1 - \lambda_o)\lambda_e & \text{LOH detected in the endometrial} \\ & \text{but not in the ovarian tumor} \\ \alpha\{\lambda_c + (1 - \lambda_c)\lambda_o\lambda_e[(1 - \rho)^2 + \rho^2]\} & \text{LOH detected in both tumors} \\ & \text{and same allele is lost in each tumor} \\ \alpha 2(1 - \lambda_c)\lambda_o\lambda_e(1 - \rho)\rho & \text{LOH detected in both tumors} \\ & \text{but a different allele is lost in each tumor,} \end{cases}$$

where  $\alpha$  is the heterozygosity of the marker and  $\rho$  is the probability that a deletion, when it occurs, affects a specific allele, either maternal or paternal. The likelihood of each outcome is calculated assuming that both tumors are derived from a common malignant precursor ( $\lambda_c \geq 0$ ) or not ( $\lambda_c = 0$ ). We define  $\lambda_c$  as the probability of LOH occurring prior to metastasis,  $\lambda_o$  as the probability that LOH occurs in the ovarian tumor after metastasis and, correspondingly,  $\lambda_e$  as the probability that LOH occurs in the endometrial tumor after metastasis. It has been estimated that, in metastatic cancers, 90% of genetic events occur before metastasis and 10% occur after metastasis (29,42,43). Therefore, for a single primary ovarian cancer with an endometrial metastasis,  $\lambda_c = 0.9 * f_o$ ,  $\lambda_o = \lambda_e = 0.1 * f_o / (1 - 0.9 * f_o)$ , where  $f_o$  and  $f_e$  are the known frequencies of LOH of that particular marker in ovarian and endometrial cancer respectively (Table 1). For independently derived primary tumors,  $\lambda_o = f_o$ ,  $\lambda_e = f_e$ , and  $\lambda_c = 0$ . To limit the number of parameters in the computations we further assumed *a priori* that deletion of either allele would be equally probable (i.e.,  $\alpha = .5$ ) and that genotyping reflects five independent events (i.e., alterations in the regions characterized at five different loci using eight different markers). The last assumption allowed us to calculate the total likelihood that the two tumors are independent or related by multiplying the likelihoods for each region. LOH frequencies for each of these regions in tumors of the ovary and endometrium derived from published literature are presented in Table 1 (32–41). For bilateral ovarian tumors, the known LOH frequencies for ovarian cancer were used.

The results of these analyses are presented as the odds in favor of a common origin, i.e., as the likelihood of a common malignant precursor divided by the likelihood of there being no common malignant precursor. This odds-in-favor value of a common origin does not depend on  $\alpha$ . We also ascertained the probability of obtaining larger odds (in favor of a common origin) assuming independently derived primary tumors, given the informativeness of the markers as observed. This was done

by simulating the observed genetic patterns 1000 times, under the assumptions described above for independent primary tumors.

## RESULTS

### Establishing the Clonal Relationship Between Synchronous Tumors From the Same Patient

We used microsatellite analysis to determine the clonal relationship between different tumor tissues from the same patient. We generated a molecular genetic fingerprint of somatic alterations based on the spectrum of LOH and MSI for each tumor. By comparing genetic fingerprints using the algorithm described in the “Patients and Methods” section, we calculated the odds in favor of the two cancers representing a single primary tumor with metastasis (odds  $>1$ ) or synchronous, independent, dual primary tumors (odds  $<1$ ). The odds obtained ranged from 0.000163 to 385 in favor of single primary tumor with related metastatic lesions.

To test the reliability of this approach for assessing clonality, we first compared genetic fingerprints in tumors from seven patients for whom there was no doubt from clinical and histopathologic diagnoses that two tumors represented either a primary tumor and a related metastatic lesion or independent primary tumors. We analyzed six case patients with a single primary tumor with a related metastatic lesion; in all case patients, genetic diagnoses confirmed that one tumor was clonally related to the other and therefore a metastasis (data not shown). We also analysed a case in which a neurofibroma had occurred incidentally in a patient with ovarian cancer; as expected, genetic analysis confirmed that the tumors had arisen independently (data not shown).

### Genetic Diagnosis in Synchronous Ovarian and Endometrial Cancers

We used the genetic fingerprinting approach above to establish the clonal relationship between tumors in 62 patients with synchronous ovarian and endometrial cancers (38 case patients), bilateral ovarian cancers (15 case patients), or bilateral ovarian and endometrial cancers (nine case patients), shown in Table 2. Examples of genetic fingerprinting using microsatellite analysis are provided in Fig. 1.

Genetic fingerprinting suggested a diagnosis of a primary tumor with metastasis in 35 (56%) case patients and a diagnosis of independent, dual primary tumors in 18 (29%) case patients; PCR failure precluded a diagnosis in seven (11%) case patients. In two (4%) case patients it was not possible to establish a clonal relationship because of extensive MSI (Table 2). We compared patient diagnoses derived from genetic data with those from the original histopathology report review. The original histopathology reports classified 26 (42%) case patients as having a single primary tumor with a related metastatic lesion and 21 (34%) case patients as having independently derived primary tumors. For five (8%) case patients, the pathologist had expressed uncertainty with respect to the relationship between the two tumors, and for 10 (16%) case patients, no opinion had been stated (Table 2).

We also compared the diagnoses obtained using the two techniques that excluded the case patients where there was uncertainty or no data with either technique (Table 3). Excluding

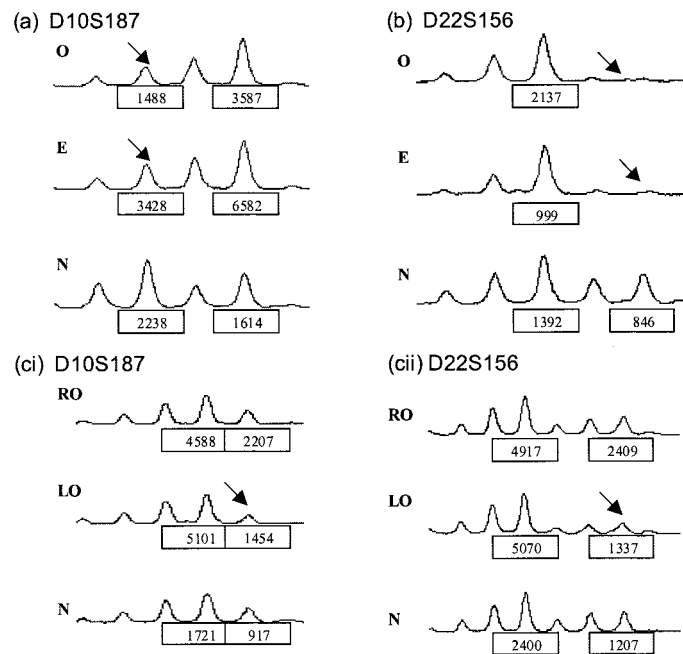


**Table 2.** Comparison between genetic and histopathologic diagnoses\*

Genetic diagnosis	Histopathologic diagnosis		
	SPWM	IDP	Uncertain or no data
Synchronous endometrial and ovarian cancers (n = 38)			
SPWM	6	11	6
IDP	3	4	1
No data	2	3	2
Synchronous bilateral ovarian cancers (n = 15)			
SPWM	6	0	2
IDP	3	0	2
No data	1	1	0
Synchronous endometrial and bilateral ovarian cancers (n = 9)			
SPWM	3	0	1
IDP	2	2	1
No data	0	0	0

\*Genetic diagnoses are based on odds in favor of primary tumor with metastasis (where odds are >1) and dual primary tumor origin (where odds are <1). SPWM = single primary tumor with related metastatic lesions; IDP = independently derived primary tumors.

the case patients for whom there was either no data or uncertainty by either technique (n = 22) and using odds of greater than 1 in favor of a genetic diagnosis of synchronous primary tumor and related metastatic lesion, we found agreement with the histopathologic diagnoses in 21 of 40 (53%) case patients and disagreement in 19 (47%) case patients. We examined



**Fig. 1.** Examples of microsatellite analysis from three different case patients with synchronous ovarian and endometrial cancers or bilateral ovarian cancer; **arrows** indicate loss of heterozygosity (LOH). The values in the boxes represent relative intensities. (a) and (b) show LOH in alleles D10S187 and D22S156, respectively at two tumor sites (O and E) in two different patients. The similar patterns suggest single primary cancers with metastases. (ci) and (cii) show alleles D10S187 and D22S156, respectively, in the same patient. There is LOH of the larger fragment in the left ovarian tumor in both alleles. This suggests a dual primary cancer. O = ovarian tumor; E = endometrial tumor; RO = right ovarian tumor; LO = left ovarian tumor; N = normal tissue.

**Table 3.** Variation in genetic diagnoses made using different statistical stringencies compared with the original histopathologic diagnoses\*

Odds	Molecular diagnosis	Histopathologic diagnosis		
		SPWM (%)	IDP (%)	% concordance
>1 or <1 (n = 40)	SPWM	15 (38)	11 (27)	53
	IDP	8 (20)	6 (15)	
>2 or <0.5 (n = 34)	SPWM	15 (44)	9 (26)	59
	IDP	5 (15)	5 (15)	
>5 or <0.2 (n = 26)	SPWM	13 (50)	4 (15)	65
	IDP	5 (20)	4 (15)	

\*Case patients with uncertain diagnoses or no data by either method were excluded. SPWM = single primary tumor with related metastatic lesions; IDP = independently derived primary tumors.

whether the differences we observed between the genetic and original histopathologic diagnosis might be due, in part, to variation in the statistical stringency with which genetic diagnoses were made. Increasing the statistical stringency did not affect the proportion of case patients that were concordant between the two methods; at odds of greater than 2, there was 59% concordance, and at odds of greater than 5, there was 65% concordance (Table 3). Therefore, the observed differences between genetic diagnoses at odds of greater than 1 and histopathologic diagnoses are real and not the result of the statistical stringency with which the genetic diagnoses were made. When odds in favor of a single primary tumor with a related metastatic lesion were greater than 1, 41% of case patients had a probability *P* value of less than .05, and when odds were less than 1, all of the case patients had a probability *P* value greater than .05. The *P* value did not influence the correlation between the original histopathology opinions and the genetic data.

Finally, we compared the frequency with which either molecular or histopathologic diagnosis was made between patients with bilateral ovarian cancer and those with ovarian and endometrial cancer. Genetic analyses classified 23 of 38 (61%) case patients with ovarian and endometrial cancer as having single primary tumors with related metastatic lesions and eight (21%) case patients as having independently derived primary tumors. For bilateral ovarian cancer, eight of 15 (53%) case patients were classified as having single primary tumors with metastatic lesions and five (33%) case patients were classified as having independent primary tumors. The concordance between genetic and histopathologic diagnoses in case patients with bilateral ovarian cancer (67%) was greater than that for case patients with endometrial and ovarian cancer (42%) (Table 2).

## DISCUSSION

In this article, we have described the development of a robust combined genetic and statistical method for establishing whether two tumors from the same patient represent independently derived primary tumors or whether one tumor is a metastasis of the other. We applied this method to the analysis of three different scenarios in gynecologic oncology: the occurrence of synchronous endometrial and ovarian tumors, bilateral ovarian tumors, and endometrial and bilateral ovarian tumors.

We have demonstrated that many of the difficulties associated with distinguishing different tumor tissues by histopathology can be overcome by using genetic characterization. To be clin-

ically relevant, any such diagnostic approach needs to be robust and performed within a short period of time; the use of microsatellite analysis fulfills these requirements. In addition, the statistical approach we have developed quantifies the likelihood that two tumors are clonally related to each other. This likelihood is dependent on the frequency with which somatic genetic alterations occur in a tumor and on differences in the pattern of genetic changes between the two tumors. This approach might be expected to cause variation in the certainty with which a diagnosis can be made; however, our findings indicate that a diagnosis made on the basis of statistical certainty affected only the proportion of case patients in which diagnoses were conclusive—it did not change any of diagnoses from that of single primary tumor with related metastatic lesions to independent primary tumors or vice versa. We used eight different microsatellite markers to assess the genetic fingerprint of each tumor. In the future, increasing the number of microsatellite markers used for the analysis is likely to increase the certainty with which a genetic diagnosis can be made without substantially increasing analytical throughput.

Perhaps the most important finding of this study was the extent of disagreement between the original histopathology opinion and the relationship between two tumors indicated by genetic analyses; there was discordance between the two approaches in approximately 40% of all case patients. The degree of discordance varied between the different clinical scenarios. In patients with bilateral ovarian cancer, there was generally a good agreement between the histopathologic and genetic diagnoses, whereas in patients with synchronous ovarian and endometrial tumors, there was less agreement between the two methods. Because the genetic technique used in these two groups was identical, it suggests that pathologists may be overdiagnosing dual primary tumors in patients with synchronous tumors of the ovary and endometrium.

The majority of bilateral ovarian tumors were diagnosed as single primary tumors with related metastatic lesions. Such a diagnosis may not be surprising for case patients presenting with disseminated pelvic and abdominal cancer; in such cases, the mechanism of metastatic spread would seem straightforward. The mechanism of metastatic spread is less clear in bilateral ovarian cancer case patients diagnosed as stage 1B, in which there is no apparent capsular extension of the disease. There were two such case patients in this study; one had multiple genetic events that were identical in both ovaries, implying a common origin. The other had a single genetic event present in the ovary only. No lymphatic channels have been described that may serve as a link between the two ovaries; thus, metastatic spread in this case may have occurred as a result of shedding of neoplastic cells from one ovary into the venous system, with subsequent preferential seeding in the contralateral ovary.

In conclusion, we have evaluated the application of genetic methods to the diagnosis of dual cancers arising in the same patient. In many case patients, genetic diagnoses substantiated the findings of histopathology, providing a diagnosis with a greater degree of certainty. More important, genetic diagnosis, as established by the algorithm described in this article, highlighted the difficulties associated with histopathologic diagnosis, suggesting that, at present, the clonal relationship between ovarian and endometrial cancers may be misdiagnosed in a substantial proportion of case patients. Furthermore, genetic diagnosis may be applicable to a broad range of clinical scenarios in

addition to those examined in this study (6,44–47). The techniques described can be incorporated into routine diagnostic histopathology to quantify the clonal relationship between multiple tumors. It is extremely useful that these genetic techniques can be successfully performed achieved with paraffin-embedded tissue. Further studies will be required to assess the wider applicability of genetic diagnosis and its possible use for improving clinical management of patients presenting with dual cancers.

## REFERENCES

- (1) Matlock DL, Salem FA, Charles EH, Savage EW. Synchronous multiple primary neoplasms of the upper female genital tract. *Gynecol Oncol* 1982;13:271–7.
- (2) Zaino RJ, Unger ER, Whitney C. Synchronous carcinomas of the uterine corpus and ovary. *Gynecol Oncol* 1984;19:329–35.
- (3) Eisner RF, Nieberg RK, Berek JS. Synchronous primary neoplasms of the female reproductive tract. *Gynecol Oncol* 1989;33:335–9.
- (4) Piura B, Glezerman M. Synchronous carcinomas of endometrium and ovary. *Gynecol Oncol* 1989;33:261–4.
- (5) Ayhan A, Yalcin OT, Tuncer ZS, Gurgan T, Kucukali T. Synchronous primary malignancies of the female genital tract. *Eur J Obstet Gynecol Reprod Biol* 1992;45:63–6.
- (6) Koness RJ, King TC, Schechter S, McLean SF, Lodowsky C, Wanebo HJ. Synchronous colon carcinomas: molecular-genetic evidence for multicentricity. *Ann Surg Oncol* 1996;3:136–43.
- (7) Huang J, Behrens C, Wistuba I, Gazdar AF, Jagirdar J. Molecular analysis of synchronous and metachronous tumors of the lung: Impact on management and prognosis. *Ann Diagn Pathol* 2001;5:321–9.
- (8) Ulbright TM, Roth LM. Metastatic and independent cancers of the endometrium and ovary: a clinicopathologic study of 34 cases. *Hum Pathol* 1985;16:28–34.
- (9) Scully RE, Richardson GS, Barlow JF. The development of malignancy in endometriosis. *Clin Obstet Gynecol* 1966;9:384–411.
- (10) Young RH, Scully RE. Metastatic tumors in the ovary: a problem-oriented approach and review of the recent literature. *Semin Diagn Pathol* 1991;8:250–76.
- (11) Scully RE, Young RH, Clement PB. Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament: atlas of tumor pathology. Silver Spring (MD): American Registry of Pathology; 1999.
- (12) Castro IM, Connell PP, Waggoner S, Rotmensch J, Mundt AJ. Synchronous ovarian and endometrial malignancies. *Am J Clin Oncol* 2000;23:521–5.
- (13) Fujikawa K, Sasaki M, Aoyama T, Itoh T, Yoshida O. Intratumoral heterogeneity in prostate cancer demonstrated by volume-weighted mean nuclear volume. *Apmis* 1997;105:322–8.
- (14) Sharifi-Salamatian V, de Roquancourt A, Rigaut JP. Breast carcinoma, intratumour heterogeneity and histological grading, using geostatistics. *Anal Cell Pathol* 2000;20:83–91.
- (15) Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 1992;51:1229–39.
- (16) Cheng PC, Goswehr JA, Kim TM, Velicescu M, Wan M, Zheng J, et al. Potential role of the inactivated X chromosome in ovarian epithelial tumor development [see comments]. *J Natl Cancer Inst* 1996;88:510–8.
- (17) Mashal RD, Fejzo ML, Friedman AJ, Mitchner N, Nowak RA, Rein MS, et al. Analysis of androgen receptor DNA reveals the independent clonal origins of uterine leiomyomata and the secondary nature of cytogenetic aberrations in the development of leiomyomata. *Genes Chromosomes Cancer* 1994;11:1–6.
- (18) Sidransky D, Frost P, Von Eschenbach A, Oyasu R, Preisinger AC, Vogelstein B. Clonal origin bladder cancer. *N Engl J Med* 1992;326:737–40.
- (19) Dinjens WN, van der Burg ME, Chadha S, Sleddens HF, Burger CW, Ewing PC. Clinical importance of molecular determinations in gynecologic patients with multiple tumors. *Cancer* 2003;97:1766–74.

- (20) Wenz HM, Dailey D, Johnson MD. Development of a high-throughput capillary electrophoresis protocol for DNA fragment analysis. *Methods Mol Biol* 2001;163:3–17.
- (21) Fujita M, Enomoto T, Wada H, Inoue M, Okudaira Y, Shroyer KR. Application of clonal analysis. Differential diagnosis for synchronous primary ovarian and endometrial cancers and metastatic cancer. *Am J Clin Pathol* 1996;105:350–9.
- (22) Pearl ML, Johnston CM, Frank TS, Roberts JA. Synchronous dual primary ovarian and endometrial carcinomas. *Int J Gynaecol Obstet* 1993;43:305–12.
- (23) Sheu BC, Lin HH, Chen CK, Chao KH, Shun CT, Huang SC. Synchronous primary carcinomas of the endometrium and ovary. *Int J Gynaecol Obstet* 1995;51:141–6.
- (24) Russell P, Bannatyne PM, Solomon HJ, Stoddard LD, Tattersall MH. Multifocal tumorigenesis in the upper female genital tract—implications for staging and management. *Int J Gynecol Pathol* 1985;4:192–210.
- (25) Annegers JF, Malkasian GD, Jr. Patterns of other neoplasia in patients with endometrial carcinoma. *Cancer* 1981;48:856–9.
- (26) Czernobilsky B, Silverman BB, Mikuta JJ. Endometrioid carcinoma of the ovary. A clinicopathologic study of 75 cases. *Cancer* 1970;26:1141–52.
- (27) Eifel P, Hendrickson M, Ross J, Ballon S, Martinez A, Kempson R. Simultaneous presentation of carcinoma involving the ovary and the uterine corpus. *Cancer* 1982;50:163–70.
- (28) Young RC. The treatment of early stage ovarian cancer. *Semin Oncol* 1995;22:76–9.
- (29) Abeln EC, Kuipers-Dijkshoorn NJ, Berns EM, Henzen-Logmans SC, Fleuren GJ, Cornelisse CJ. Molecular genetic evidence for unifocal origin of advanced epithelial ovarian cancer and for minor clonal divergence. *Br J Cancer* 1995;72:1330–6.
- (30) Pejovic T, Heim S, Mandahl N, Elmfors B, Furgyik S, Floderus UM, et al. Bilateral ovarian carcinoma: cytogenetic evidence of unicentric origin. *Int J Cancer* 1991;47:358–61.
- (31) Diaz-Cano SJ, Brady SP. DNA extraction from formalin-fixed, paraffin-embedded tissues: protein digestion as a limiting step for retrieval of high-quality DNA. *Diagn Mol Pathol* 1997;6:342–6.
- (32) Cliby W, Ritland S, Hartmann L, Dodson M, Halling KC, Keeney G, et al. Human epithelial ovarian cancer allelotype. *Cancer Res* 1993;53:2393–8.
- (33) Fujino T, Risinger JI, Collins NK, Liu FS, Nishii H, Takahashi H, et al. Allelotype of endometrial carcinoma. *Cancer Res* 1994;54:4294–8.
- (34) Gabra H, Langdon SP, Watson JE, Hawkins RA, Cohen BB, Taylor L, et al. Loss of heterozygosity at 11q22 correlates with low progesterone receptor content in epithelial ovarian cancer. *Clin Cancer Res* 1995;1:945–53.
- (35) Imamura T, Arima T, Kato H, Miyamoto S, Sasazuki T, Wake N. Chromosomal deletions and K-ras gene mutations in human endometrial carcinomas. *Int J Cancer* 1992;51:47–52.
- (36) Jones MH, Koi S, Fujimoto I, Hasumi K, Kato K, Nakamura Y. Allelotype of uterine cancer by analysis of RFLP and microsatellite polymorphisms: frequent loss of heterozygosity on chromosome arms 3p, 9q, 10q, and 17p. *Genes Chromosomes Cancer* 1994;9:119–23.
- (37) Kiechle-Schwarz M, Bauknecht T, Wienker T, Walz L, Pfeleiderer A. Loss of constitutional heterozygosity on chromosome 11p in human ovarian cancer. Positive correlation with grade of differentiation. *Cancer* 1993;72:2423–32.
- (38) Okamoto A, Sameshima Y, Yamada Y, Teshima S, Terashima Y, Terada M, et al. Allelic loss on chromosome 17p and p53 mutations in human endometrial carcinoma of the uterus. *Cancer Res* 1991;51:5632–5.
- (39) Osborne RJ, Leech V. Polymerase chain reaction allelotyping of human ovarian cancer. *Br J Cancer* 1994;69:429–38.
- (40) Peiffer SL, Herzog TJ, Tribune DJ, Mutch DG, Gersell DJ, Goodfellow PJ. Allelic loss of sequences from the long arm of chromosome 10 and replication errors in endometrial cancers. *Cancer Res* 1995;55:1922–6.
- (41) Pieretti M, Powell DE, Gallion HH, Case EA, Conway PS, Turker MS. Genetic alterations on chromosome 17 distinguish different types of epithelial ovarian tumors. *Hum Pathol* 1995;26:393–7.
- (42) Sasatomi E, Finkelstein SD, Woods JD, Bakker A, Swalsky PA, Luketich JD, et al. Comparison of accumulated allele loss between primary tumor and lymph node metastasis in stage II non-small cell lung carcinoma: implications for the timing of lymph node metastasis and prognostic value. *Cancer Res* 2002;62:2681–9.
- (43) Shenson DL, Gallion HH, Powell DE, Pieretti M. Loss of heterozygosity and genomic instability in synchronous endometrioid tumors of the ovary and endometrium. *Cancer* 1995;76:650–7.
- (44) Einbeigi Z, Meis-Kindblom JM, Kindblom LG, Wallgren A, Karlsson P. Clustering of individuals with both breast and ovarian cancer—a possible indicator of BRCA founder mutations. *Acta Oncol* 2002;41:153–7.
- (45) Curtin JP, Barakat RR, Hoskins WJ. Ovarian disease in women with breast cancer. *Obstet Gynecol* 1994;84:449–52.
- (46) van Oijen MG, Leppers Vd Straat FG, Tilanus MG, Slootweg PJ. The origins of multiple squamous cell carcinomas in the aerodigestive tract. *Cancer* 2000;88:884–93.
- (47) Louhelainen J, Wijkstrom H, Hemminki K. Allelic losses demonstrate monoclonality of multifocal bladder tumors. *Int J Cancer* 2000;87:522–7.

## NOTES

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