

Loss of Heterozygosity, Microsatellite Instability and *TP53* Gene Status in Ovarian Carcinomas

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Abstract. *Background: Microsatellite instability (MSI) and loss of heterozygosity (LOH) are frequent events in ovarian carcinogenesis; however, little is known as to their clinical significance and association with other molecular lesions. Materials and Methods: Twelve microsatellite markers for MSI and LOH analysis were used in 64 ovarian carcinomas with known TP53 mutational status. The clinical importance of molecular alterations was evaluated in a uniform subgroup of patients treated with platinum-based regimens. Results: LOH was detected in order of frequency at 17p13.3 (D17S926, 79%), 17p13.1 (TP53 locus, 69%), 13q14 (RB, 60%), 3p21 (D3S1611, 32.5%), 8q21 (D8S1811, 22%), 11p14/13 (D11S904, 19%), 10qter (D10S197, 13%) and 2p16-21 (D2S123, 11%). LOH at the RB1 locus showed association with LOH at the TP53 locus ($p=0.01$). Platinum sensitivity was associated with heterozygosity at the TP53 locus ($p=0.006$). Only one tumor displayed microsatellite instability in one marker (RB) only. Conclusion: Our results suggest that LOH at the 17p D17S926 locus in ovarian cancer is an earlier molecular event than LOH at the TP53 locus. Inactivation of TP53 and RB1 genes may have a synergistic effect in ovarian tumorigenesis.*

Microsatellite instability (MSI) and/or loss of heterozygosity (LOH) at different chromosomal arms are implicated in ovarian cancer development; however, they are seldom

analyzed in the context of other molecular lesions, such as *TP53* gene mutations, and little is known about their biological and clinical significance in ovarian cancer patients.

MSI is caused by defects in the mismatch repair system (MMR), usually by genetic or epigenetic inactivation of *hMLH1* and *hMSH2* genes. MSI has been reported in 0-77% of ovarian carcinomas (most commonly in 10-20%) (1-5). Higher frequency of microsatellite instability was observed in endometrioid, mucinous or clear cell types (3, 6-8) and in clinical stage I (6). MSI is regarded as a component of the molecular pathways underlying development of borderline tumors and mainly of better differentiated ovarian carcinomas.

hMLH1 and *hMSH2* proteins recognize and repair mismatched DNA and play a role in detection of DNA adducts that are formed as a result of cisplatin treatment. Defects in the MMR pathway may be associated with ovarian cancer resistance to chemotherapy. It has been demonstrated that mismatch repair-deficient cells characterized by microsatellite instability were resistant to various cytotoxic agents including cisplatin (9-11).

On the other hand, inactivation of the *TP53* tumor suppressor pathway, usually due to *TP53* mutation and/or LOH at the *TP53* locus (17p13.1) underlies a development of the majority of poorly differentiated ovarian carcinomas. LOH occurs frequently as a second hit (after mutation) also in a process of inactivation of other tumor suppressor genes, such as *BRCA1*, *BRCA2*, *RB1* and *NBS1* (at 17q21, 13q12.3, 13q14.1-14.2, 8q21-24, respectively). A consequent deficiency of DNA repair may lead to genomic instability and some losses may occur further by chance. In ovarian carcinoma, LOH is a common phenomenon, mainly on 3p, 5q, 6, 9p, 11, 13, 16q, 17, 18 and 22 (12, 13). Among these, there are loci with high LOH frequency (e.g. 9q22-31, 3p22-24, 11p15.5-3) without there being any knowledge of linked genes important for ovarian carcinogenesis.

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To date, *TP53* gene mutation is the best defined somatic lesion in poorly differentiated ovarian carcinomas. The analysis of the LOH pattern with respect to *TP53* gene status may help to find alterations contributing to tumor development driven by mutant *TP53*. Among many reports on LOH in ovarian carcinomas, only single studies analyzed associations between *TP53* mutations and LOH at regions other than 17p13.1 (*TP53* locus) (14-16). Such coincidence was found in one study only, for 5q (14). Associations between LOH at the *TP53* locus and LOH at other loci (e.g. *BRCA1*, 3p, Xq, 9q) were reported more frequently (16-19).

Allelic losses at specific regions, as well as total LOH rate per tumor may influence tumor response to chemotherapy as reported for different types of cancers (20-24). In ovarian tumors, a predictive value of LOH at specific chromosomal regions has not been demonstrated; however, there are only single reports on this subject (19, 25).

The aim of our study was to evaluate the rate of microsatellite instability (as a marker of MMR inactivation) and the frequency and pattern of LOH in ovarian carcinomas with respect to *TP53* gene status. Another aim of the study was to evaluate the significance of these molecular events for tumor response to platinum-based chemotherapy.

Materials and Methods

Tumors and patients for molecular and clinical analysis. Molecular analysis was performed on 64 carcinomas, including 59 ovarian and five disseminated peritoneal carcinomas of Mullerian type. Tissues came from primary laparotomy prior to chemotherapy. Tumor samples were snap frozen in liquid nitrogen and stored at -80°C . Normal tissue frozen or embedded in paraffin was available for each case.

The tumors were classified histologically according to the criteria of the World Health Organization (26). There were 41 serous, 4 mucinous, 6 endometrioid, 3 clear cell, 2 transitional cell type, 6 undifferentiated and one unclassified carcinoma, and one undifferentiated sarcoma. Histological grade was evaluated on a 4-grade scale according to Broders' criteria (27). Four tumors were well differentiated (G1, 6%), ten showed moderate differentiation (G2, 16%), 28 were poorly differentiated (G3, 44%) and 22 were mostly or completely undifferentiated (G4, 34%). The tumors were staged according to the criteria of the International Federation of Gynecologists and Obstetricians (28). All tumors have been previously studied for *TP53* gene mutations (29).

Complete clinicopathological data (including a treatment information and the follow-up) was available for the subgroup of 31 patients (30). The patients were treated with cisplatin or carboplatin and cyclophosphamide, or else with addition of doxorubicin (as previously described, 30). The platinum-sensitive group (PS) had disease-free survival (DFS) longer than or equal to six months according to the definition given by Christian and Trimble (31). Other tumors (partial remission, progression, no change), as well as the complete remission (CR) group with DFS shorter than six months were described as resistant to cisplatin (30,

Table 1. Loss of heterozygosity (LOH) and informativity of all markers studied.

Marker	Chromosome	Locus	LOH	Informative cases
D17S926	17p13.3		34 (79%)	43 (67%)
TP53	17p13.1	<i>TP53</i>	40 (69%)	58 (91%)
TP53-Alu	17p13.1	<i>TP53</i>	20 (69%)	29 (47.5%)
D17S261	17p11.2-12		15 (62.5%)	24 (37.5%)
RB	13q14	<i>RB1</i>	30 (60%)	50 (79%)
D3S1611	3p21	<i>MLH1</i>	13 (32.5%)	40 (64.5%)
D8S1811	8q21	<i>NBS1</i>	5 (22%)	23 (36.5%)
D11S904	11p14/13		6 (19%)	31 (49%)
D10S197	10qter		6 (13%)	46 (73%)
D2S123	2p16-21	<i>MSH2</i>	5 (11%)	44 (71%)
BAT26	2p16-21	<i>MSH2</i>	-	0
BAX	19q13.3-13.4	<i>BAX</i>	-	0

31). There were 15 platinum-sensitive and 16 platinum-resistant carcinomas. This group was almost uniform and consisted of advanced FIGO stage (III or IV) poorly differentiated carcinomas. There were no mucinous or clear cell tumors.

DNA was isolated with the use of QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). Frozen sections were cut from each specimen and were evaluated by a pathologist (JK) for the relative content of non-tumor tissue.

Marker selection. MSI and LOH can be concomitantly detected by PCR amplification of microsatellites (DNA simple repetitive sequences). Twelve polymorphic markers at 8 different chromosomes were selected (Table I). Marker selection was based on the following criteria: the majority of markers were previously reported to show a high percentage of instability in tumors, and some of them displayed additionally high levels of allelic losses (D10S197, D11S904). The susceptibility to instability might be dependent on the particular type of repetitive motif, therefore markers with four different repeat types were studied: mononucleotide (poly-A BAT26 and poly-G BAX), tetranucleotide (RB), pentanucleotide (TP53-Alu) and simple or complex dinucleotide repeats (other markers). In addition, BAT26 has high specificity in detecting tumors with a high level of microsatellite instability (MSI-H), whereas D2S123 is highly sensitive in recognizing tumors with the high and low level of microsatellite instability (MSI-L). Four markers were chosen for the short arm of chromosome 17 because of the high frequency of *TP53* mutations in our tumors (29). The majority of selected markers are linked to known genes (Table I).

PCR-based fluorescence detection. Microsatellite sequences were amplified using one 5'-end fluorescently labeled and one unlabeled primer for each locus. The forward primer of each pair was labeled with one of three dyes (6-FAM, TET, HEX) and the fourth dye (TAMRA) was reserved for the size standard.

The loci were amplified in 6.5 μL volume by a polymerase chain reaction (PCR). The PCR mixture was prepared according to the protocol provided with a Perkin Elmer AmpliTaq Gold PCR kit (Applied Biosystems Division, Foster City, CA, USA).

Amplification was carried out for 36 cycles on a programmable thermal cycler (Biometra, Gottingen, Germany) with denaturation at 94°C, annealing at 53-61°C (depending on the marker) and extension at 72°C, each for 30 s. Due to different amplification efficiencies, aliquots of the PCR products were diluted in sterile distilled water to achieve comparable strength between paired tumor and non-tumor DNA, and to prevent off-scale data.

A volume of 0.5 µL of each PCR product was then added to 1.5 µL of loading mix (formamide/blue dextran/EDTA and GeneScan-500 TAMRA size standard, Applied Biosystems). This mix was denatured for 5 min at 95°C, cooled on ice and loaded onto a 5% Long Ranger/6 M urea gel (BioWhittaker Molecular Applications, Rockland, ME, USA) in an Abi Prism 377 Sequencer. Up to 7 markers distinguishable by size or fluorescent dye were run on a single gel line for electrophoretic separation. The data were collected automatically and analyzed using GeneScan Analysis Software (Applied Biosystems).

Criteria for evaluation of LOH and MSI. Only cases with normal DNA heterozygous for a given locus were informative for LOH analysis. The LOH value was calculated using the following algebraic formula recommended by Applied Biosystems' GeneScan Reference Guide: $LOH\ value = N2:N1/T2:T1$ where T1 and N1 are peak heights of the shorter allele and T2 and N2 of the longer allele for the tumor (T) and normal (N) sample, respectively. On the basis of calculated LOH value the results were divided into two groups: i) LOH-positive cases with significant loss of the shorter ($LOH\ value \leq 0.5$) or longer allele ($LOH \geq 1.5$); and ii) LOH-negative cases with an LOH value between 0.5 and 1.5. These criteria were established to reliably evaluate LOH in samples with non-malignant cell content up to 50% (Applied Biosystems' GeneScan Reference Guide). Microsatellite instability was identified when additional bands were noticed in tumor DNA compared with normal DNA.

Statistical analysis. Associations among genetic alterations and between genetic alterations and platinum sensitivity were studied by Fisher's exact test. Associations between LOH and clinicopathological parameters (histological type and grade, FIGO stage) were evaluated in a multivariate logistic regression model. Important factors were selected using backward selection technique, where factors not significant at 0.1 were stepwise removed from the model. All calculations were carried out using the STATA 7.0 program (Stata Corporation, College Station, TX, USA).

Results

Ten out of 12 markers were informative for LOH analysis. TP53 was the most informative marker with 91% of informative cases, while BAT26 and BAX markers were non-informative (Table I).

LOH analysis on the short arm of chromosome 17. The polymorphic markers used in our study represent three regions of the short arm of chromosome 17. TP53 and TP53-Alu are located exactly in the TP53 gene locus, 17p13.1. D17S261 is localized at the more centromeric region 17p11.2-12. The marker D17S926 represents the most distal region, 17p13.3.

Fifty-two out of 63 informative tumors (82.5%) exhibited LOH in at least one locus at 17p; among these, 43 (68%) had loss at all informative 17p loci. The frequency of LOH at 17p was the highest at the distal locus D17S926 (17p13.3, 79%) and decreased toward the centromeric region (Table I). There were tumors with LOH at D17S926 only, while no tumor heterozygous for this locus had LOH at more proximal regions. The two markers linked exactly to the TP53 locus presented with equal frequency of LOH, at 69%. However, the total number of tumors with LOH at each of the two loci was different due to different number of informative cases (Table I). The more proximally located marker (D17S261) had a minimally lower rate of LOH (62.5%) and LOH at this locus was always accompanied by LOH at TP53 and/or TP53-Alu.

LOH at other loci studied. The frequency of LOH at other loci studied is presented in Table I. LOH beyond 17p was associated with LOH at 17p ($p=0.001$). In the whole group, only two tumors presented with LOH beyond 17p only (at single marker, RB or D11S904). One was a clear cell carcinoma with LOH at D11S904, the other one was a poorly differentiated serous carcinoma with LOH at RB only.

The tetranucleotide repeat in the 20th intron of the *RB1* gene was the second most frequently altered chromosomal region. LOH was detected in 30 of 50 informative cases (60%). There was an association between LOH at 17p and LOH at the *RB1* locus. Twenty-five out of 27 tumors with LOH at RB (93%) also exhibited LOH at the TP53 locus (17p11.2-13.1) ($p=0.01$). Only two tumors with LOH at RB (7%) retained heterozygosity at the TP53 locus, while 12 tumors had LOH at the TP53 locus without LOH at RB.

Tumors without LOH. In nine tumors (14%) no LOH at informative loci studied was detected. Interestingly, among these tumors were 3 disseminated peritoneal carcinomas of Mullerian type, two of which had a TP53 gene mutation. Three tumors without LOH were poorly differentiated. Clinicopathological characteristics of these tumors are presented in Table II. Some of them had relatively high stromal cell contamination (scc); however, among tumors with LOH detected, the normal cell content ranged from 0 to 60%.

TP53 mutational status and LOH data. Fifty-three out of 64 tumors (83%) had a TP53 gene mutation. Tumors with TP53 mutation had a higher incidence of LOH at 17p11.2-13.1 than tumors without a mutation (84% versus 36%, respectively, $p=0.0028$). An association of TP53 mutation with LOH at D17S926 (distal locus) was stronger: 92% of the tumors with TP53 mutation showed concomitant loss of heterozygosity at D17S926 ($p=0.0001$). An association of LOH at the *RB1* locus with TP53 mutation ($p=0.0046$) appeared stronger than with LOH at the TP53 locus.

Table II. Histopathological and clinical profile of tumors without loss of heterozygosity (LOH) and/or without TP53 gene mutation.

Number	Histological type	Tumor grade	FIGO stage	LOH	TP53 mutation	% of informative markers	scc
290*	Serous	2	IIIC	None	+	70	30%
98*	Serous	3	IV	None	+	90	30-40%
114	Mucinous	1	IA	None	+	70	60-70%
30	Serous	2	IV	None	None	60	≤20%
270	Serous	2	IIIC	None	None	30	<1%
130	Serous	2	IIIB	None	None	60	15-20%
370*	Serous	3	IIIC	None	None	40	30-40%
294	Endometrioid	1	IA	None	None	80	10%
283	Sarcoma	3	IA	None	None	80	50%
37	Serous	2	IIIC	TP53, D8S1811	None	50	10-20%
264	Serous	4	IIIC	TP53, RB	None	60	25%
212	Mucinous	1	IA	TP53	None	50	50-60%
208	Clear cell	2	IC	D17S261, TP53, TP53-Alu	None	70	5-10%
94	Clear cell	3	IC	D11S904	None	80	<1%

*Peritoneal; scc = stromal cell contamination.

LOH and clinicopathological parameters. Tumor grade was the most significant parameter associated with LOH status. Occurrence of LOH at 17p11.2-13.1, D17S926, RB and also beyond chromosome 17p was associated with high tumor grade ($p=0.016$, 0.009 , 0.01 and 0.001 , respectively). The probability of occurrence of LOH at at least one locus was higher in tumors of high grade ($p=0.001$, odds ratio (OR)=27.4, 95% confidence interval for OR 3.6-210.5) and of serous/undifferentiated/other types ($p=0.045$, OR=31.1, 95% C.I. for OR 1.08-893).

LOH and platinum sensitivity. Analysis of the clinical significance of LOH with respect to platinum sensitivity was performed in a clinicopathologically uniform group of 31 tumors. Since the number of LOH at particular markers beyond the chromosome 17p was low, statistical analysis was performed with regard to 17p regions only. Platinum sensitivity was associated with heterozygosity at the TP53 locus (17p11.2-13.1) ($p=0.006$, Fisher's exact test). All tumors which retained heterozygosity at this locus were sensitive to cisplatin. TP53 gene mutational status (presence vs. no mutation; missense type vs. other status) showed no association with platinum sensitivity.

Microsatellite instability. The ovarian tumors studied did not present microsatellite instability, except one. Tumor 271 (serous carcinoma, FIGO IIIC, grade 3) had MSI at the RB marker only (Figure 1). Interestingly, MSI was not detected in 11 tumors without TP53 mutation in which it could be expected. Histopathological and clinical characteristics of the latter tumors are presented in Table II.

Discussion

Among novel findings in our study are the high frequency of LOH at the D17S926 locus, associations between TP53 gene status (LOH at TP53 locus, mutations) and LOH at the RB1 locus, and the predictive value of LOH at the TP53 locus.

Our results confirm a high frequency of LOH at the short arm of chromosome 17 in ovarian carcinomas. In the literature, LOH at 17p was observed in 42-83% (12, 13, 32), while LOH at TP53 locus in 25-80% of ovarian carcinomas (13, 15, 17, 32-36). Similarly to our results, LOH at the more telomeric 17p13.3 region (with markers different from D17S926 used in our study) was observed more frequently than LOH at the TP53 locus (up to 86%) (32, 33, 37). A declining frequency of LOH from the telomeric to the centromeric region of 17p might suggest higher instability of telomeric regions in the LOH process. Following this hypothesis, White *et al.* also analyzed telomeric markers on other chromosomal arms (in breast and lung tumors) and did not confirm this rule (38). Thus, the LOH at the D17S926 locus appears to be an event which takes place earlier than LOH at any other locus studied in our analysis and it is highly associated with TP53 gene mutation.

The region defined by the D17S926 marker was studied in ovarian tumors by one group only (39). In that study the D17S926 marker was distal to a common region of allelic loss. D17S926 was studied more frequently in other tumors (thyroid, esophageal, breast, lung), and was frequently located in a minimal region of deletion (38, 40,

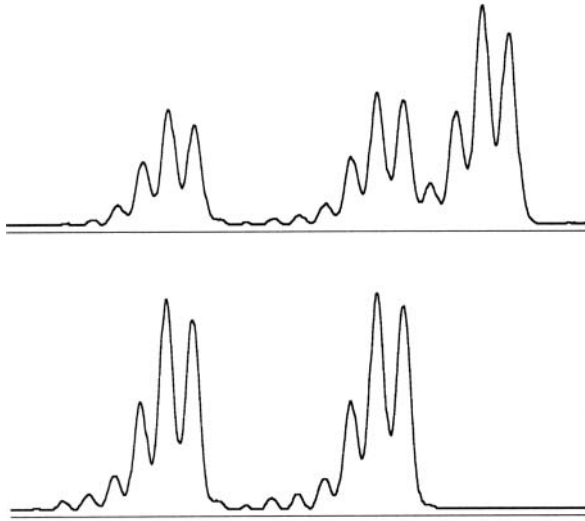


Figure 1. Microsatellite instability at the RB marker. The top graph represents tumor DNA and the bottom graph represents normal DNA. In comparison with heterozygous normal DNA (two alleles visible), in tumor DNA additional alleles are seen.

41). A high frequency of LOH at the D17S926 marker might suggest that this chromosomal region contains a tumor suppressor gene. The region defined by marker D17S926 was reported as a putative locus for genes for several congenital disorders (42-44) and for different tumors (38, 40, 41, 45). Moreover, a few genes (*e.g.* *GEMIN4*, *NXN*, *HCCS1* and *C17orf25*) have been mapped close to the region detected by the D17S926 marker. One of these genes, *HCCS1* is a candidate tumor suppressor gene for hepatic carcinoma (46). In almost all tumors studied by this group, *HCCS1* gene mutations were accompanied by LOH at D17S926 (46). In the study by Xiao *et al.* all tumors with LOH at intragenic polymorphic loci in *HCCS1* had also LOH at the D17S926 marker (47). Thus, the D17S926 locus should be further explored in ovarian carcinomas.

The second most altered chromosomal region in ovarian carcinomas in our study was the *RB1* locus at 13q14. In the literature, the frequency of LOH at the *RB1* in ovarian carcinomas ranges between 23.5-67% (mean 44%) (12, 13, 17, 48). In contrast to other studies on this subject (13, 16, 17), we found an association between LOH at the *RB1* locus and LOH at the *TP53* locus, and this was the only association between allelic losses on the different chromosomes studied. Concurrent inactivation of *RB1* and *TP53* genes led to ovarian tumorigenesis in the mouse (49). These observations may suggest that inactivation of *RB1* and *TP53* genes has a synergistic effect in ovarian carcinogenesis.

According to data from cell lines, efficiency of platinum-based chemotherapy is highly dependent on apoptosis induced by *TP53*. In clinical studies on ovarian cancer, the issue of the predictive value of *TP53* alterations remains controversial (50-53), while a predictive value of LOH at 17p13 has not been found (19, 25); however, in one study on 30 ovarian cancer patients, tumor response to cisplatin-based chemotherapy was influenced by the total LOH rate per tumor (25). There are single reports on tumors of other organs describing deletions at 17p as a predictor of resistance to chemotherapy (22-24). We are the first to demonstrate such a clinical importance of LOH at 17p13 in ovarian carcinoma patients treated with cisplatin-based regimens; however, our results should be confirmed on larger groups.

We did not find microsatellite unstable tumors in our group of ovarian carcinomas. Particularly interesting is the lack of MSI in tumors in which MSI could be expected such as endometrioid, mucinous and clear cell carcinomas, as well as FIGO stage I tumors, or those without *TP53* mutation, or any LOH detected. This result is supported by the positive staining of hMLH1 and hMSH2 proteins in a larger group of our ovarian carcinomas (233 tumors, data not shown). The lack of hMLH1 or hMSH2 expression was frequently reported as a feature of MSI tumors, including ovarian carcinomas (36, 54-57).

Our study is comparable with other analyses as to the group size and the number of markers studied. Our set contains markers with proven usefulness for detecting MSI and/or LOH (1, 3, 7, 58). In particular, the D3S1611 marker (intragenic to the *hMLH1* gene) was proven sensitive in detecting MSI in ovarian tumors (12-13%) (1, 3). In some reports, markers linked to the *TP53* locus exhibited the highest frequency of MSI among all markers studied (7, 58).

In the literature, the frequency of MSI in ovarian tumors ranges from none to 77% (usually 10-20%) (1-3, 5, 8, 36). Some authors described no or very little microsatellite unstable ovarian tumors, as in our study (4, 59-62). It appears that in ovarian carcinomas, the mutator phenotype is not a common alternative to a deficient *TP53* pathway.

Summarizing, in accord with data from the literature, our study shows that inactivation of 17p appears to play a dominant role in ovarian carcinogenesis. The major role is attributed to deficient *TP53*, nevertheless, an analysis of the D17S926 locus could provide additional insight. Our results suggest also "a cooperation" between dysfunctional *TP53* and *RB1*.

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References

- 1 Sood AK, Holmes R, Hendrix MJ and Buller RE: Application of the National Cancer Institute international criteria for determination of microsatellite instability in ovarian cancer. *Cancer Res* 61: 4371-4374, 2001.
- 2 Lavarino C, Pilotti S, Oggionni M, Gatti L, Perego P, Bresciani G, Pierotti MA, Scambia G, Ferrandina G, Fagotti A, Mangioni C, Lucchini V, Vecchione F, Bolis G, Scarfone G and Zunino F: *P53* gene status and response to platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma. *J Clin Oncol* 18: 3936-3945, 2000.
- 3 Ohwada M, Suzuki M, Saga Y and Sato I: DNA replication errors are frequent in mucinous cystadenocarcinoma of the ovary. *Cancer Genet Cytogenet* 117: 61-65, 2000.
- 4 Helleman J, van Staveren IL, Dinjens WN, van Kuijk PF, Ritstier K, Ewing PC, van der Burg ME, Stoter G and Berns EM: Mismatch repair and treatment resistance in ovarian cancer. *BMC Cancer* 6: 201, 2006.
- 5 Geisler JP, Goodheart MJ, Sood AK, Holmes RJ, Hatterman-Zogg MA and Buller RE: Mismatch repair gene expression defects contribute to microsatellite instability in ovarian carcinoma. *Cancer* 98: 2199-2206, 2003.
- 6 King BL, Carcangiu ML, Carter D, Kiechle M, Pfisterer J, Pfleiderer A and Kacinski BM: Microsatellite instability in ovarian neoplasms. *Br J Cancer* 72: 376-382, 1995.
- 7 Fujita M, Enomoto T, Yoshino K, Nomura T, Buzard GS, Inoue M and Okudaira Y: Microsatellite instability and alterations in the hMSH2 gene in human ovarian cancer. *Int J Cancer* 64: 361-366, 1995.
- 8 Gras E, Catusas L, Arguelles R, Moreno-Bueno G, Palacios J, Gamallo C, Matias-Guiu X and Prat J: Microsatellite instability, *MLH-1* promoter hypermethylation, and frameshift mutations at coding mononucleotide repeat microsatellites in ovarian tumors. *Cancer* 92: 2829-2836, 2001.
- 9 Fink D, Nebel S, Aebi S, Zheng H, Cenni B, Nehme A, Christen RD and Howell SB: The role of DNA mismatch repair in platinum drug resistance. *Cancer Res* 56: 4881-4886, 1996.
- 10 Fink D, Zheng H, Nebel S, Norris PS, Aebi S, Lin TP, Nehme A, Christen RD, Haas M, MacLeod CL and Howell SB: *In vitro* and *in vivo* resistance to cisplatin in cells that have lost DNA mismatch repair. *Cancer Res* 57: 1841-1845, 1997.
- 11 Aebi S, Kurdi-Haidar B, Gordon R, Cenni B, Zheng H, Fink D, Christen RD, Boland CR, Koi M, Fishel R and Howell SB: Loss of DNA mismatch repair in acquired resistance to cisplatin. *Cancer Res* 56: 3087-3090, 1996.
- 12 Cliby W, Ritland S, Hartmann L, Dodson M, Halling KC, Keeney G, Podratz KC and Jenkins RB: Human epithelial ovarian cancer allelotype. *Cancer Res* 53: 2393-2398, 1993.
- 13 Yang-Feng TL, Han H, Chen KC, Li SB, Claus EB, Carcangiu ML, Chambers SK, Chambers JT and Schwartz PE: Allelic loss in ovarian cancer. *Int J Cancer* 54: 546-551, 1993.
- 14 Tavassoli M, Steingrimsdottir H, Pierce E, Jiang X, Alagöz M, Farzaneh F and Campbell IG: Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by *TP53* mutation and identifies a tumour suppressor gene locus at 5q13.1-21. *Br J Cancer* 74: 115-119, 1996.
- 15 Okada S, Tsuda H, Takarabe T, Yoshikawa H, Taketani Y and Hirohashi S: Allelotype analysis of common epithelial ovarian cancers with special reference to comparison between clear cell adenocarcinoma with other histological types. *Jpn J Cancer Res* 93: 798-806, 2002.
- 16 Manderson EN, Presneau N, Provencher D, Mes-Masson AM and Tonin PN: Comparative analysis of loss of heterozygosity of specific chromosome 3, 13, 17, and X loci and *TP53* mutations in human epithelial ovarian cancer. *Mol Carcinog* 34: 78-90, 2002.
- 17 Villeneuve JB, Silverman MB, Alderete B, Cliby WA, Li H, Croghan GA, Podratz KC and Jenkins RB: Loss of markers linked to *BRCA1* precedes loss at important cell cycle regulatory genes in epithelial ovarian cancer. *Genes Chromosomes Cancer* 25: 65-69, 1999.
- 18 Choi C, Cho S, Horikawa I, Berchuck A, Wang N, Cedrone E, Jhung SW, Lee JB, Kerr J, Chenevix-Trench G, Kim S, Barrett JC and Koi M: Loss of heterozygosity at chromosome segment Xq25-26.1 in advanced human ovarian carcinomas. *Genes Chromosomes Cancer* 20: 234-242, 1997.
- 19 Byrom J, Mudaliar V, Redman CW, Jones P, Strange RC and Hoban PR: Loss of heterozygosity at chromosome 9q22-31 is a frequent and early event in ovarian tumors. *Int J Oncol* 24: 1271-1277, 2004.
- 20 Grundei T, Vogelsang H, Ott K, Mueller J, Scholz M, Becker K, Fink U, Siewert JR, Hofler H and Keller G: Loss of heterozygosity and microsatellite instability as predictive markers for neoadjuvant treatment in gastric carcinoma. *Clin Cancer Res* 6: 4782-4788, 2000.
- 21 Thiessen B, Maguire JA, McNeil K, Huntsman D, Martin MA and Horsman D: Loss of heterozygosity for loci on chromosome arms 1p and 10q in oligodendroglial tumors: relationship to outcome and chemosensitivity. *J Neurooncol* 64: 271-278, 2003.
- 22 Blons H, Cabelguenne A, Carnot F, Laccourreye O, de Waziers I, Hamelin R, Brasnu D, Beaune P and Laurent-Puig P: Microsatellite analysis and response to chemotherapy in head-and-neck squamous-cell carcinoma. *Int J Cancer* 84: 410-415, 1999.
- 23 Goto A, Kanda H, Ishikawa Y, Matsumoto S, Kawaguchi N, Machinami R, Kato Y and Kitagawa T: Association of loss of heterozygosity at the *p53* locus with chemoresistance in osteosarcomas. *Jpn J Cancer Res* 89: 539-547, 1998.
- 24 Mehes G: Chromosome abnormalities with prognostic impact in B-cell chronic lymphocytic leukemia. *Pathol Oncol Res* 11: 205-210, 2005.
- 25 Nakayama S, Nakayama K, Takebayashi Y, Hata K, Fujiwaki R, Fukumoto M and Miyazaki K: Allelotypes as potential prognostic markers in ovarian carcinoma treated with cisplatin-based chemotherapy. *Int J Mol Med* 11: 621-625, 2003.
- 26 Russell P: Surface epithelial-stromal tumors of the ovary. In: Blaustein's Pathology of the Female Genital Tract. Kurman RJ (ed.). Berlin Heidelberg, Springer-Verlag, pp. 705-782, 1994.
- 27 Barber HR, Sommers SC, Synder R and Kwon TH: Histologic and nuclear grading and stromal reactions as indices for prognosis in ovarian cancer. *Am J Obstet Gynecol* 121: 795-807, 1975.
- 28 Creasman WJ: Announcement, FIGO stages 1988, Revisions. *Gynecol Oncol* 35: 125-127, 1989.
- 29 Dansonka-Mieszkowska A, Ludwig AH, Kraszewska E and Kupryjanczyk J: Geographical variations in *TP53* mutational spectrum in ovarian carcinomas. *Ann Hum Genet* 70: 594-604, 2006.

- 30 Kupryjanczyk J, Madry R, Plisiecka-Hałasa J, Bar J, Kraszewska E, Ziolkowska I, Timorek A, Stelmachow J, Emerich J, Jedryka M, Pluzanska A, Rzepka-Gorska I, Urbanski K, Zielinski J and Markowska J: TP53 status determines clinical significance of ERBB2 expression in ovarian cancer. *Br J Cancer* 91: 1916-1923, 2004.
- 31 Christian MC and Trimble EL: Salvage chemotherapy for epithelial ovarian carcinoma. *Gynecol Oncol* 55: S143-150, 1994.
- 32 Sato T, Saito H, Morita R, Koi S, Lee JH and Nakamura Y: Allelotype of human ovarian cancer. *Cancer Res* 51: 5118-5122, 1991.
- 33 Phillips N, Ziegler M, Saha B and Xynos F: Allelic loss on chromosome 17 in human ovarian cancer. *Int J Cancer* 54: 85-91, 1993.
- 34 Okamoto A, Sameshima Y, Yokoyama S, Terashima Y, Sugimura T, Terada M and Yokota J: Frequent allelic losses and mutations of the p53 gene in human ovarian cancer. *Cancer Res* 51: 5171-5176, 1991.
- 35 Launonen V, Mannermaa A, Stenback F, Kosma VM, Puistola U, Huusko P, Anttila M, Bloigu R, Saarikoski S, Kauppila A and Winqvist R: Loss of heterozygosity at chromosomes 3, 6, 8, 11, 16, and 17 in ovarian cancer: correlation to clinicopathological variables. *Cancer Genet Cytogenet* 122: 49-54, 2000.
- 36 Watanabe Y, Koi M, Hemmi H, Hoshai H and Noda K: A change in microsatellite instability caused by cisplatin-based chemotherapy of ovarian cancer. *Br J Cancer* 85: 1064-1069, 2001.
- 37 Phillips NJ, Ziegler MR, Radford DM, Fair KL, Steinbrueck T, Xynos FP and Donis-Keller H: Allelic deletion on chromosome 17p13.3 in early ovarian cancer. *Cancer Res* 56: 606-611, 1996.
- 38 White GR, Stack M, Santibanez-Koref M, Liscia DS, Venesio T, Wang JC, Helms C, Donis-Keller H, Betticher DC, Altermatt HJ, Hoban PR and Heighway J: High levels of loss at the 17p telomere suggest the close proximity of a tumour suppressor. *Br J Cancer* 74: 863-870, 1996.
- 39 Schultz DC, Vanderveer L, Berman DB, Hamilton TC, Wong AJ and Godwin AK: Identification of two candidate tumor suppressor genes on chromosome 17p13.3. *Cancer Res* 56: 1997-2002, 1996.
- 40 Farrand K, Delahunt B, Wang XL, McIver B, Hay ID, Goellner JR, Eberhardt NL and Grebe SK: High resolution loss of heterozygosity mapping of 17p13 in thyroid cancer: Hurthle cell carcinomas exhibit a small 411-kilobase common region of allelic imbalance, probably containing a novel tumor suppressor gene. *J Clin Endocrinol Metab* 87: 4715-4721, 2002.
- 41 Huang J, Hu N, Goldstein AM, Emmert-Buck MR, Tang ZZ, Roth MJ, Wang QH, Dawsey SM, Han XY, Ding T, Li G, Giffen C and Taylor PR: High frequency allelic loss on chromosome 17p13.3-p11.1 in esophageal squamous cell carcinomas from a high incidence area in northern China. *Carcinogenesis* 21: 2019-2026, 2000.
- 42 Balciuniene J, Johansson K, Sandgren O, Wachtmeister L, Holmgren G and Forsman K: A gene for autosomal dominant progressive cone dystrophy (CORD5) maps to chromosome 17p12-p13. *Genomics* 30: 281-286, 1995.
- 43 Greenberg J, Goliath R, Beighton P and Ramesar R: A new locus for autosomal dominant retinitis pigmentosa on the short arm of chromosome 17. *Hum Mol Genet* 3: 915-918, 1994.
- 44 Krebsova A, Hamm H, Karl S, Reis A and Hennies HC: Assignment of the gene for a new hereditary nail disorder, isolated congenital nail dysplasia, to chromosome 17p13. *J Invest Dermatol* 115: 664-667, 2000.
- 45 Orellana C, Hernandez-Marti M, Martinez F, Castel V, Millan JM, Alvarez-Garijo JA, Prieto F and Badia L: Pediatric brain tumors: loss of heterozygosity at 17p and TP53 gene mutations. *Cancer Genet Cytogenet* 102: 93-99, 1998.
- 46 Zhao X, Li J, He Y, Lan F, Fu L, Guo J, Zhao R, Ye Y, He M, Chong W, Chen J, Zhang L, Yang N, Xu B, Wu M, Wan D and Gu J: A novel growth suppressor gene on chromosome 17p13.3 with a high frequency of mutation in human hepatocellular carcinoma. *Cancer Res* 61: 7383-7387, 2001.
- 47 Xiao W, Park CK, Park JY, Lee JH, Kim HS, Cho YG, Kim CJ, Ahn YM, Song YH, Lee SH, Yoo NJ, Lee JY and Park WS: Genetic alterations of the HCCS1 gene in Korean hepatocellular carcinoma. *APMIS* 111: 465-473, 2003.
- 48 Gras E, Pons C, Machin P, Matias-Guiu X and Prat J: Loss of heterozygosity at the RB-1 locus and pRB immunostaining in epithelial ovarian tumors: a molecular, immunohistochemical, and clinicopathologic study. *Int J Gynecol Pathol* 20: 335-340, 2001.
- 49 Flesken-Nikitin A, Choi KC, Eng JP, Shmidt EN and Nikitin AY: Induction of carcinogenesis by concurrent inactivation of p53 and Rb1 in the mouse ovarian surface epithelium. *Cancer Res* 63: 3459-3463, 2003.
- 50 Reles A, Wen WH, Schmider A, Gee C, Runnebaum IB, Kilian U, Jones LA, El-Naggar A, Minguillon C, Schonborn I, Reich O, Kreienberg R, Lichtenegger W and Press MF: Correlation of p53 mutations with resistance to platinum-based chemotherapy and shortened survival in ovarian cancer. *Clin Cancer Res* 7: 2984-2997, 2001.
- 51 Righetti SC, Della Torre G, Pilotti S, Menard S, Ottone F, Colnaghi MI, Pierotti MA, Lavarino C, Cornarotti M, Oriana S, Bohm S, Bresciani GL, Spatti G and Zunino F: A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. *Cancer Res* 56: 689-693, 1996.
- 52 Niwa K, Itoh M, Murase T, Morishita S, Itoh N, Mori H and Tamaya T: Alteration of p53 gene in ovarian carcinoma: clinicopathological correlation and prognostic significance. *Br J Cancer* 70: 1191-1197, 1994.
- 53 Iba T, Kigawa J, Kanamori Y, Itamochi H, Oishi T, Simada M, Uegaki K, Naniwa J and Terakawa N: Expression of the c-myc gene as a predictor of chemotherapy response and a prognostic factor in patients with ovarian cancer. *Cancer Sci* 95: 418-423, 2004.
- 54 Cai KQ, Albarracin C, Rosen D, Zhong R, Zheng W, Luthra R, Broaddus R and Liu J: Microsatellite instability and alteration of the expression of hMLH1 and hMSH2 in ovarian clear cell carcinoma. *Hum Pathol* 35: 552-559, 2004.
- 55 Liu J, Albarracin CT, Chang KH, Thompson-Lanza JA, Zheng W, Gershenson DM, Broaddus R and Luthra R: Microsatellite instability and expression of hMLH1 and hMSH2 proteins in ovarian endometrioid cancer. *Mod Pathol* 17: 75-80, 2004.
- 56 Singer G, Kallinowski T, Hartmann A, Dietmaier W, Wild PJ, Schraml P, Sauter G, Mihatsch MJ and Moch H: Different types of microsatellite instability in ovarian carcinoma. *Int J Cancer* 112: 643-646, 2004.

- 57 Rosen DG, Cai KQ, Luthra R and Liu J: Immunohistochemical staining of hMLH1 and hMSH2 reflects microsatellite instability status in ovarian carcinoma. *Mod Pathol* 19: 1414-1420, 2006.
- 58 Hickey KP, Boyle KP, Jepps HM, Andrew AC, Buxton EJ and Burns PA: Molecular detection of tumour DNA in serum and peritoneal fluid from ovarian cancer patients. *Br J Cancer* 80: 1803-1808, 1999.
- 59 Bozzetti C, Bortesi B and Merisio C: Loss of heterozygosity (LOH) in ovarian cancer. *Int J Gynaecol Obstet* 85: 294-295, 2004.
- 60 Iwabuchi H, Sakamoto M, Sakunaga H, Ma YY, Carcangiu ML, Pinkel D, Yang-Feng TL and Gray JW: Genetic analysis of benign, low-grade, and high-grade ovarian tumors. *Cancer Res* 55: 6172-6180, 1995.
- 61 Osborne RJ and Leech V: Polymerase chain reaction allelotyping of human ovarian cancer. *Br J Cancer* 69: 429-438, 1994.
- 62 Pieretti M, Cavalieri C, Conway PS, Gallion HH, Powell DE and Turker MS: Genetic alterations distinguish different types of ovarian tumors. *Int J Cancer* 64: 434-440, 1995.

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