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Original Paper

Molecular Characterisation of a Panel of Human Ovarian Carcinoma Xenografts

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In a panel of 16 human ovarian tumours transplanted in nude mice, the expression of genes involved in cell cycle regulation and in response to drug treatment were characterised. In the 16 tumours analysed we could not detect overexpression of *Erb-B2* oncogene while expression of *MDR1* mRNA was not detected in 11/15 samples and was low in 4/15 tumours. Only three tumours had mutations in the *p53* gene exons 5–8 and one of these mutations did not result in any amino acid alteration. The levels of mRNA for *cyclins A*, *D1* and *E* were heterogeneous with some tumours expressing high levels and others not expressing them at all. The same was found for the cyclin dependent kinases (CDK) *CDK2* and *CDK4* and for CDK inhibitors *p21/WAF1*, *p27/KIP1* and *p16/CDKN2*. Two genes belonging to the nucleotide excision repair, *ERCC1* and *ERCC3* were detectable in all the samples examined, as were the genes *MGMT* and *MAG*, also involved in DNA repair. The data indicate a heterogeneity in the expression of genes considered to be involved in the cellular responses to cytotoxic drug treatment and indicate the possibility of using these tumour models to test specifically molecules with a defined mechanism of action.

Key words: ovarian cancer, xenografts, cell cycle proteins, DNA repair, drug resistance
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INTRODUCTION

THE SUCCESS of chemotherapy of ovarian cancer has been hampered by the advanced stage of the disease at the time of diagnosis and by the development of resistance to chemotherapy [1].

For drugs which are commonly used in ovarian cancer, such as platinum co-ordination complexes, alkylating agents, doxorubicin and taxanes, resistance mechanisms appear to be multifactorial. In ovarian tumour cell lines, the reported resistance mechanisms include: (1) decreased drug accumulation, which can be due to enhanced expression of drug transporter proteins such as P-glycoprotein (MDR), MDR-associated protein (MRP) and lung resistance protein (LRP); (2) increased detoxification, mostly due to binding of drugs to glutathione, catalysed by glutathione-S-transferase (GSTπ); (3) increased DNA repair, which can occur by enhanced expression of DNA repair genes such as O^6-alkyl-guanine-methyl-transferase (*MGMT*), 3-methyladenine DNA glycosylase (*MAG*) and proteins involved in nucleotide excision repair such as ERCC1 and ERCC3; and (4) changes in the level of the drug's target, for example decrease in DNA topoisomerase I and II enzymes (TOPO I and TOPO II) [2].

Moreover, there is increasing evidence on the key role of *p53* in controlling the cellular response to DNA damage, either by increasing the transcription of genes involved in growth arrest and repair, such as *WAF1* and *GADD45*, or by inducing pro-apoptotic genes such as *BAX* [3, 4]. The *p53* gene is often mutated in human cancer [5, 6] where the control mechanisms are, therefore, defective.

Further defects in the regulation of cell cycle progression are thought to be one common feature of transformed cells [7–9]. The key enzymes involved in cell cycle regulation are a group of serine/threonine kinases known as cyclin-dependent kinases (CDKs), which bind to regulatory subunits, the cyclins. CDKs are activated and inactivated at specific time

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points during the cell cycle in order to control cell division. An important role in controlling the correct cell cycle progression is also played by CDK inhibitors, such as p21, p27, p16, p18 and p57, small proteins which bind and inactivate/destroy the CDK–cyclin complexes [10].

We report here a panel of 16 human ovarian xenografts established *in vivo* in nude mice that were characterised for their growth characteristics and for the expression of genes possibly involved in drug resistance and in cell cycle regulation.

MATERIALS AND METHODS

Animals

Female NCr nu/nu mice were obtained from the Division of Cancer Treatment, National Cancer Institute, Animal Production Colony, Frederick, Maryland, U.S.A. and were used when 8–10 weeks old and weighing 21 ± 2 g. The mice were housed in air-filtered laminar flow cabinets and manipulated according to aseptic procedures.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC council directive 86/609, OJL 358, 1, 12 December 1987; NIH guide for the care and use of laboratory animals, NIH publication no. 85–23, 1985).

Establishment of human ovarian carcinoma (HOC) xenografts

P-HOC8, Y-HOC8, HOC10, HOC18, HOC22 were established and maintained in nude mice as described previously [11, 12]. HOC76, HOC94/2, HOC78, HOC79, HOC84, HOC106, HOC107, HOC109 and HOC110 are newly established xenografts directly derived from patients with adenocarcinoma of the ovary. COR(T) was established in nude mice from *in vitro* cell culture [13], OVCAR-3 was obtained from the Medicine Branch, National Cancer Institute, Bethesda, Maryland, U.S.A., as frozen ascite derived from an *in vivo* model.

Tumour specimens (primary tumours, metastasis, ascitic or pleural effusions) from patients were obtained via laparotomy or paracentesis or thoracentesis. Tumour tissue was processed as described elsewhere [11, 14]. Briefly, the tissue was dissected, freed of any necrotic tissue, cut into fragments and injected into the flanks of nude mice (subcutaneous (s.c.) transplant). Ascitic or pleural effusions were centrifuged, the cells were washed and resuspended in HBSS and injected into the peritoneal cavity of nude mice (intraperitoneal (i.p.) transplant).

Tumour cells from *in vitro* cultures growing as monolayers in their appropriate culture medium were detached, washed and resuspended in HBSS and s.c. injected into the flanks of nude mice.

Routinely, the established HOC xenografts were maintained for serial passages in nude mice by transplantation of 10^7 tumour cells i.p. or tumour fragments s.c. All xenografts were tested within 10 passages in nude mice.

All specimens from patient samples were classified by routine histopathological examination according to the FIGO classification for ovarian tumours. The histotype of the xenografts growing in nude mice was compared with that found in the patient's tumour and no differences were observed. Patient origin and growth behaviour of the xenografts are reported in Tables 1 and 2.

Drug treatment

Cisplatin (CDDP), provided by Bristol-Myers Squibb (Wallingford, Connecticut, U.S.A.), was dissolved in 0.9% NaCl and given intravenously (i.v.) every 4 days for 12 days (Q4×3), at the maximum tolerated dose of 4 mg/kg.

On s.c. growing tumours, treatment started when the tumours reached a mean weight of 150 ± 50 mg. On i.p. growing tumours, treatment started on a day corresponding to 25% of the median survival time (MST) of the mice. The presence of a tumour in the peritoneal cavity at the beginning of treatment was confirmed by cytohistological analysis of three additional mice per group as described previously [12].

Treatment evaluation

Mice were weighed three times a week to evaluate drug-induced toxicity, and body weight changes were recorded during treatments.

Table 1. *Clinical characteristics of ovarian cancer patients*

Tumour model	Specimen source*	Histological type	Grade	Stage	Previous treatment†	Response†
HOC18	Primary tumour	Serous	G3	IV	EPI, CTX	Partial response
HOC106	Recurrence	Serous	G3	IIIc	PAC, paclitaxel (6)	Partial response
HOC107	Recurrence	Serous	G3	IIIc	PAC, paclitaxel (6)	Partial response
HOC109	Peritoneal metastasis	Endometrioid	G2	Ic	CBDCA, paclitaxel (7)	Progression
P-HOC8	Pleural effusion	Serous	G3	IV	None	–
Y-HOC8	Ascitic effusion	Serous	G3	IV	CBDCA, CDDP, CTX, EPI	Remission
HOC10	Ascitic effusion	Serous	G3	III	None	–
HOC22	Ascitic effusion	Serous	G3	III	CBDCA, RX	Progression
HOC76	Ascitic effusion	Serous	G3	III	PAC, paclitaxel (3)	Progression
HOC78	Ascitic effusion	Endometrioid	G2	III	CDDP, PAC, paclitaxel (6)	Progression
HOC79	Ascitic effusion	Endometrioid	G2	III	CDDP, DOX + CTX, MTX + IF + paclitaxel (1)	Progression
HOC84	Pleural effusion	Serous	G3	IIIc	CDDP, paclitaxel (2)	Progression
HOC94/2	Ascitic effusion	Serous	G2	Ia	PAC, paclitaxel (6)	Progression
HOC110	Ascitic effusion	Serous	G2		CBDCA	Progression
COR(T)	Peritoneal metastasis	Serous	G3	III	CP, PAC, CDDP, paclitaxel (6)	Partial response
OVCAR-3	*In vitro* cell culture	Frozen ascites from NCI			PAC	

*Origin of tumour cells injected into nude mice. †At time of injection into nude mice. CDDP, cisplatin; CTX, cyclophosphamide; EPI, epirubicin; CBDCA, carboplatin; RX, radiotherapy; DOX, doxorubicin; CP, cyclophosphamide + cisplatin; PAC, cisplatin + doxorubicin + cyclophosphamide (the number of cycles performed are in parentheses).

Table 2. Biological characteristics of the different xenografts

	Nude mice behaviour			
Tumour line	Growth site	Doubling time*	MST (range)†	CDDP sensitivity‡
HOC18	s.c.	11.2 ± 2.4	n.t.	++
HOC106	s.c.	12.2 ± 2	n.t.	n.t.
HOC107	s.c.	37.7 ± 10	n.t.	n.t.
HOC109	s.c.	8.2 ± 2	n.t.	+++
P-HOC8	i.p.	n.t.	63 (34–134)	+
Y-HOC8	i.p.	n.t.	66 (33–136)	–
HOC10	i.p.	n.t.	75 (46–110)	–
HOC22	i.p.	n.t.	55 (24–124)	+
HOC76	i.p.	n.t.	67 (46–169)	n.t.
HOC78	s.c.	20.1 ± 5.1	n.t.	+++
HOC79	i.p.	n.t.	71 (38–96)	–
HOC84	s.c.	12 ± 3	n.t.	n.t.
HOC94/2	s.c.	10 ± 2	n.t.	++
HOC110	i.p.	n.t.	92 (90–108)	++
COR(T)	s.c.	29 ± 4.5	n.t.	n.t.
OVCAR-3	i.p.	n.t.	52 (48–69)	+

n.t., not tested; i.p. intraperitoneal; s.c., subcutaneous; ILS, increase in lifespan; T/C, median relative tumour weight (treated)/median relative tumour weight (control). * ± Standard deviation (for s.c. tumours). †Median survival time (for i.p. growing tumours). ‡CDDP (cisplatin) was given at 4 mg/kg, every 4 days three times intravenously. –, inactive (ILS < 40%; T/C > 50%); +, minimal activity (40% < ILS < 75%; T/C < 50%); ++, moderate activity (75% < ILS < 100%; T/C < 40%) ; +++, good activity (100% < ILS < 150%; T/C < 25%); ++++, optimal activity (ILS > 50% or complete remissions; T/C < 10%).

For s.c. growth, the diameters of the tumours were measured twice a week in two dimensions with a caliper and estimates of tumour weights were calculated as (length×width^2)/2. Changes in tumour weights from the start of treatment were calculated as relative tumour weights and the median of these values in the control and the treated groups (%T/C) was used to calculate treatment efficacy. Results are expressed as described in Table 2.

For i.p. growth, mice were monitored twice a week for tumour formation (abdominal distension) in the peritoneal cavity. Animals were sacrificed when they became moribund and the day of sacrifice was considered as a survival time. The end point used for assessing activity was the increase in lifespan (%ILS), calculated as 100×[(MST of treated group – MST of control group)/MST of control group], and the results expressed as described in Table 2. The absence of a tumour in 'cured' mice was confirmed by cytohistological examination, as described previously [12].

Northern blot analysis

Total RNAs were prepared using the guanidium thiocyanate–caesium chloride method as described previously [15]. Total RNA (10–15 μg) from ovarian cancer xenografts was fractionated by electrophoresis on formaldehyde 1% agarose gels, blotted and immobilised on to Nylon membranes (GeneScreen Plus, NEN, Boston, MA, U.S.A.) and were hybridised with specific 32P-labelled probes, using the RedyPrime kit (Amersham, Milan, Italy). Hybridisation was performed in 50% formamide, 10% dextran sulphate, 1% sodium dodecyl sulphate (SDS) and 1 M NaCl for 16–18 h at 42°C with 5-10×10^{5}cpm/ml of labelled probes.

Filters were washed twice with 2×SSC at room temperature and once with 2×SSC–1%SDS at 65°C and autoradiographed. Membranes were stripped and rehybridised with α-actin cDNA which was used as a control for densitometric analysis.

Band intensity was quantified using a densitometer and normalised by calculating the ratio between the values obtained for each mRNA and the corresponding α-actin mRNA. mRNA analysis was performed for some genes at different passages of the tumours. The results obtained were not significantly different.

Probes

The probes for MRP, WAF1 and GADD45 were obtained by reverse transcription–polymerase chain reaction (RT–PCR), using oligonucleotides spanning the coding region derived from the published human sequences [16–18] and subsequent subcloning in pMOS blue T-vector (Amersham). The identities of the three genes were verified by sequencing.

Other probes were: the plasmid pBShTOP2 containing the entire coding region of h-TOP2 (supplied by Dr J.C. Wang, Department of Biochemistry and Molecular Biology, Harvard University, U.S.A.); the 4300-bp fragment of YEpGAL1-hTOP1 (supplied by P. Benedetti, Institute of Cell Biology of CNR, Rome, Italy); the 1.3 kb *Eco*RI-*Sal*I fragment of pc DR 1.3 of the h-*MDR1* gene [19]; the 725-bp *Eco*RI-*Hind*III fragment of h GSTπ (supplied by Dr J.A. Moscow, National Institute of Health, Bethesda, Maryland, U.S.A.); the 1.2-kb fragment isolated from PUC 9-MPG plasmid (gift from Professor B. Kaina, University of Mainz, Germany); the 0.8-kb fragment pKT100 for MGMT (gift from Dr S. Mitra, University of Texas, Texas, U.S.A.); the 748-bp *Kpn*I-*Stu*I fragment of Erb-B2/NEU [20]; the 1.1-kb fragment from pF12012 for ERCC1 (kindly provided by Dr J.H.J. Holÿmaker, Erasmus University, Rotterdam, The Netherlands); the 28-kb fragment from pCDI for ERCC3; the 229-bp fragment from p18CL7M for h-BAX (provided by S.J. Korsmeyer, Washington University, St Louis, Missouri, U.S.A.); the 1.3-kb *Not*I fragment of mouse cyclin D1 cDNA and the 980-bp from pK4PCRCDK4 for CDK4 (provided from Dr C. Sherr, St. Jude Children's Research Hospital,

Table 3. Sequence of p53 *primers used for polymerase chain reaction–single-strand conformational polymorphism analysis*

Exon	Sense primer (5′-3′)	Antisense primer (5′-3′)
5	TTCCTCTTCCTGCAGTACTC	ACCCTGGGCAACCAGCCCTGT
6	CACTGATTGCTCTTAGGT	AGTTGCAAACCAGACCTGAG
7	TGGGCGACAGAGCGAGATTCCATCT	AGTATGGAAGAAATCGGTAAGAGGT
8	CCTATCCTGAGTAGTGGTAA	TTGTCCTGCTTGCTTACCTC

Memphis, TN, U.S.A.); the *Eco*RI fragment of h-cyclin E and the Bgl II fragment of h-cyclin A (provided by Dr S.I. Reed, Department of Molecular Biology MB7, The Scripps Research Institute. La Jolla, California, U.S.A.); the 2-kb *Xho*I fragment of h-CDK2 (provided by American Type Culture Collection, Rockville, Maryland, U.S.A.); the 1.5-kb fragment from pBSHISK-hp27 for p27 (supplied by Joan Massague, Sloan-Kettering Institute, New York, U.S.A.); the 800-bp *Eco*RI-*Xho*I fragment of h-p16 (supplied by Dr D. Blach, Cold Spring Harbour Laboratory. New York, U.S.A.).

PCR–single-strand conformational polymorphism (SSCP) analysis

Exons 5–8 of the *p53* gene were examined by PCR–SSCP. The primers used for amplification are reported in Table 3.

PCR conditions consisted of one cycle at 95°C for 2 min, 40 cycles at 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, followed by one cycle at 72°C for 10 min. Reactions took place in a 20 µl volume of 1×PCR buffer containing 1 µM primers, 200 µM dNTPs, 0.4 U Taq polymerase and 0.4 µCi of a 32P-dCTP. The PCR products were mixed with an equal volume of 0.1% SDS–10 mM ethylene diamine tetra acetic acid (EDTA) solution, and then with an equal volume of loading buffer, heated at 95°C for 5 min and loaded on to 6% polyacrylamide gels containing 5% glycerol. When aberrantly migrating bands were revealed in SSCP analysis, the PCR products were sequenced using a PCR sequencing kit (Amersham).

Western blot analysis

Frozen tumour samples were pulverised, then lysed in a solution containing 0.1 M sodium chloride, 10 mM Tris–HCl pH 7.6, 1 mM EDTA, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 100 µg/ml phenylmethylsulphonyl fluoride (PMSF), centrifuged for 5 min at 12 000 g at 4°C and the protein concentration measured using the BioRad protein assay (BioRad, Milan, Italy); 100 µg of proteins were mixed with an equal volume of 2×loading buffer (0.1 M Tris–HCl pH 6.8, 4% SDS, 0.2 M dithiothreitol, 0.2% bromophenol blue and 20% glycerol), boiled, size-fractionated through SDS/15% polyacrylamide gels and blotted onto nitrocellulose filters (Schleyer & Schull, Dossen, Germany). The filters were hybridised with polyclonal antibodies against p16 and cyclin E (Santa Cruz Biotechnology, Heidelberg, Germany) and revealed using the ECL system (Amersham).

RESULTS

p53 *Mutations*

Using PCR–SSCP analysis, we screened for the presence of mutations in exons 5–8 of the *p53* gene in all the tumours. The samples showing aberrant migration by SSCP were sequenced to confirm and assess precisely the mutation. Of the 16 tumours examined, three had mutations in the *p53* gene, one in exon 5, and two in exon 6 (Table 4). One of the three mutations found did not result in an amino acid change, thus 14 of 16 samples had no mutations in the four highly conserved exons (5–8) of *p53*.

Expression of cell cycle regulatory genes

Figures 1 and 2 show the mRNA expression of CDKs, cyclins and CDK inhibitors in the 16 HOC tumour cell lines examined.

CDK2 mRNA was not detectable by Northern analysis in two (P-HOC8 and HOC10) of 16 tumour lines, while *CDK4* mRNA levels were measurable in all the samples tested. Some variation in the levels between the different tumour samples were observed. The highest levels of both *CDK2* and *CDK4* were found in HOC76, although considering all the samples together, there was no evident correlation between the levels of the two kinases.

Cyclin A was detected in all tumour lines and at relatively higher levels in HOC18, HOC76 and OVCAR-3. *Cyclin D1* expression was not detected in OVCAR-3 and was only weakly positive in P-HOC8, HOC10 and HOC22. Heterogeneous expression levels were found for *cyclin E*. In fact, HOC10, HOC18 and HOC79 differed from the other lines by having very high levels of mRNA expression, whereas it was not detected in HOC106, HOC109 and HOC110 and detected at only low levels in HOC107, HOC76, HOC84 and OVCAR-3. *WAF1* expression was not detected in four of 16 tumour lines, *KIP1* in three of 14 lines tested and *CDKN2* in four of 15. In HOC18, neither *KIP1* nor *WAF1* mRNA levels were detected, while in HOC109, *CDKN2* and *KIP1* were concomitantly undetectable.

Apart from *WAF1*, two other genes regulated by *p53* (*BAX* and *GADD45*) were studied. Expression of the pro-apoptotic *BAX* gene was not detected in Y-HOC8, HOC10, and COR(T) and was at the limit of detection in HOC84 (Figure 2).

GADD45 expression was not detected in five tumour lines, while very high levels were found in HOC22 and P-HOC8 which had levels more than 4 and 10 times higher, respectively, than all the other tumour samples tested.

For cyclin E and p16, we also analysed, in some tumours, expression at the protein level by Western blotting. Figure 3 indicates that a reasonable correlation between mRNA and protein levels was found.

Table 4. Mutations of p53 *gene found in ovarian carcinoma xenografts*

Xenograft	Exon	Codon	Change in	
			Nucleotide	Amino acid
HOC18	6	213	CGA–CGG	Arg–Arg
HOC78	5	173	GTG–GGG	Val–Gly
HOC79	6	193	CAT–CGT	His–Arg

Expression of genes involved in drug resistance

The *MDR1* gene, responsible for the multidrug resistance phenotype, was not detected by Northern analysis, in 11 of 15 xenografts and no evidence of high overexpression of this gene was observed in the four positive tumours (data not shown). The level of expression was very low in the four positive samples (HOC109, HOC79, HOC110 and OVCAR-3) and did not allow quantitative analysis. MRP mRNA was highly expressed in P-HOC8, COR(T) and HOC94/2, but was not detected in HOC22 (Figure 2). All the tumour samples were positive for the expression of two genes belonging to the NER family, i.e. *ERCC1* and *ERCC3* (Figure 2). These two genes were heterogeneously expressed in the different tumours and their expression was unrelated, i.e. tumour samples expressing high levels of *ERCC1* did not express high levels of *ERCC3*.

Two other genes involved in DNA repair genes, *MGMT* and *MAG*, were positively expressed in all the xenografts tested (Figure 2). *GSTμ* gene was expressed by all xenografts tested and reached high levels in P-HOC8, HOC10, HOC79 and COR(T).

For the DNA topoisomerase genes, *TOPO I* was detected in all the samples tested, while *TOPO II* was not detected in three of the 16 samples and was present at very low levels in two other samples (Figure 2). HOC10 showed the highest

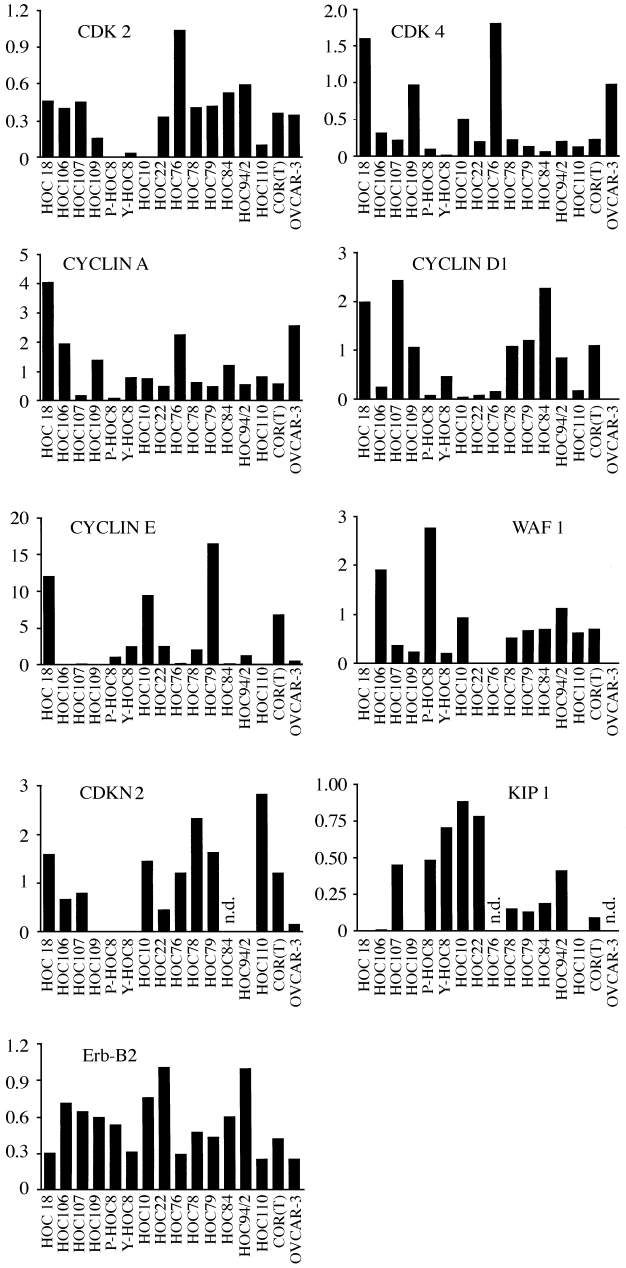

Figure 1. mRNA expression level of cell cycle regulating genes in the different human ovarian carcinoma xenografts. The values represent the ratio between the densitometric value of each mRNA transcript and that of α-actin. For details see Materials and Methods. n.d., not determined.

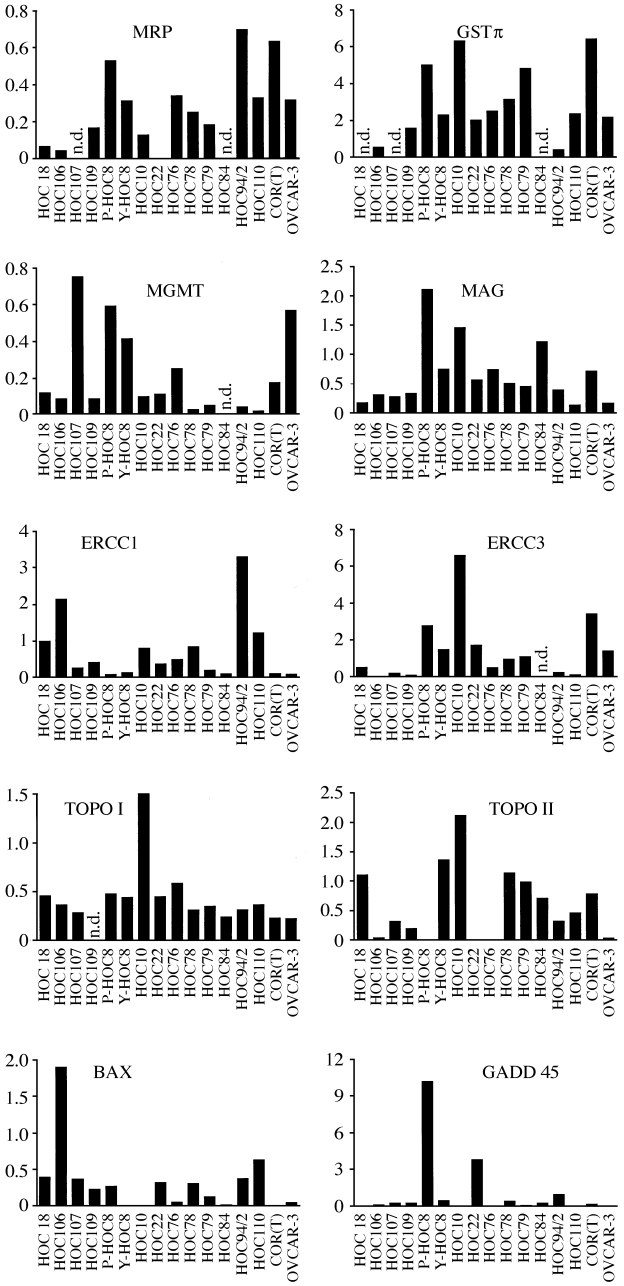

Figure 2. mRNA expression level of genes involved in DNA repair and response to drug treatment in 16 human ovarian carcinoma xenografts . The values represent the ratio between the densitometric value of each mRNA transcript and that of α-actin. For details see Materials and Methods. n.d., not determined.

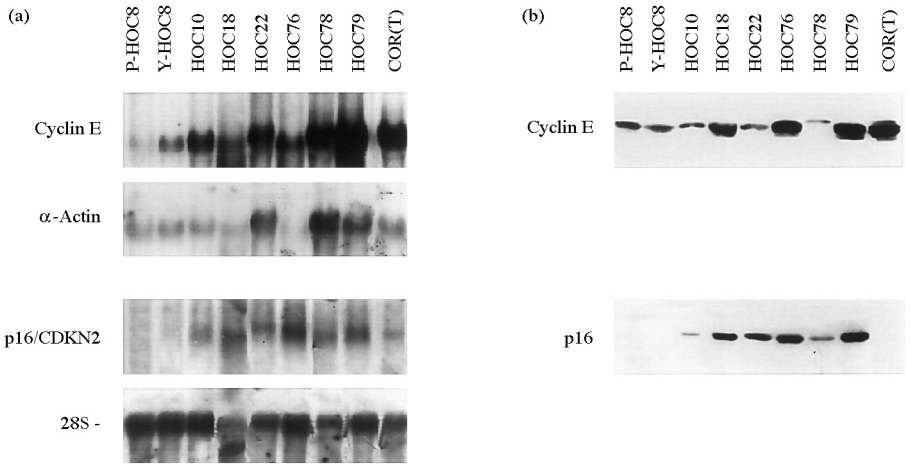

Figure 3. (a) mRNA and (b) protein levels of *p16* and *cyclin E* in nine human ovarian carcinoma xenografts. α-Actin and 28S are reported (for *cyclin E* and *p16*, respectively) to show loading differences.

expression of both *TOPO I* and *TOPO II*. *Erb-B2* oncogene was expressed in all 16 samples examined but no overexpression (as compared with expression levels in the SKOV3 cell line–data not shown) was found (Figure 1).

DISCUSSION

Although chemotherapeutic agents used for advanced ovarian cancer (i.e. cisplatin or analogues, alkylating agents, taxanes and anthracyclines) produce tumour regression in a large fraction of patients, their impact on patients' survival is still limited [1]. The discovery and development of new therapies is, therefore, urgently needed [21]. One of the crucial aspects of the development of new anticancer drugs is the choice of the experimental models used to test and select potential candidate molecules for clinical investigations. Several laboratories have recently obtained ovarian cancer xenografts which appear to maintain some of the relevant features of the *in vivo* tumour from which they are derived (e.g. pathological or genetic features, degree of sensitivity to drugs). The molecular characterisation of ovarian xenografts reported so far in the literature is limited, so our report of the characterisation of 16 human ovarian tumours implanted in nude mice aims to obtain a type of fingerprint of the molecular alterations as a basis for testing the antitumour activity of new molecules with specific mechanisms of action.

An important question is whether these xenografts are representative of the human tumour from which they were derived. In general, the mRNA expression of the analysed genes remained stable when it was measured in tumour tissue taken at different passage numbers. Moreover, the data presented appear to be in good agreement with those recently reported in fresh ovarian cancers, at least for *MDR1*, *p53* and *Erb-B2* expression. In fact, the *MDR1* gene has been reported to be amplified in a small percentage of patients with ovarian cancer [22] and the results from our study concur with this, with low detection of mRNA for *MDR1* in our xenografts.

No overexpression of the *Erb-B2* oncogene was found in these tumours and these results are in agreement with previous observations reporting a very low percentage of amplification/overexpression of this gene in ovarian cancers [23].

Finally, the *p53* gene was found mutated in three of 16 samples (19%), resulting in a percentage similar to that reported in the literature [5, 24]. Considering that the presence of *p53* mutations and/or *Erb-B2* overexpression can be associated with a 'more malignant' tumour, our data suggest that the cell lines established *in vivo* do not arise from selection, at least considering the above-mentioned parameters, of the more malignant cells present in the tumour of origin.

The results of genes involved in DNA repair/resistance suggest that, in these samples, no gross deletions/rearrangements are present. In fact for most of the genes, we detected a mRNA of the expected size in all the samples evaluated. *TOPO II* and *ERCC3* genes were not detected in a few tumour samples, but this may have been due to low sensitivity of the Northern blotting analysis, rather than to a true lack of expression.

We found no correlations between the expression of the various genes analysed. There is increasing evidence supporting a role for proteins involved in cell cycle regulation in malignant transformation [9, 25]. In fact, deletions, mutations or overexpression of almost all the genes involved in the cell cycle control (cyclins, CDKs and CDK inhibitors) are invariably observed in the majority of human tumours. In particular, correlation between a worst prognosis and/or survival and the overexpression of cyclins E and D1 has been reported [26–28]. Recently, a strong correlation between survival and *KIP1* expression was found in breast and colorectal cancer [29–31].

In the xenografts analysed, cyclin E overexpression was found in three tumours (HOC10, HOC18 and HOC79) without any evident correlation with an increased doubling time or lower MST. The same was found for *KIP1* which was not detected in three tumour lines (HOC18, HOC109 and HOC110). The HOC18 tumour, in particular, had very high levels of cyclins E and D but *KIP1* and *WAF1* were absent, a condition which should lead to a more aggressive tumour [27, 30]. It may be that mRNA expression does not reflect expression at the protein level or that other parameters, such as response to chemotherapy, should be taken into account to detect a possible correlation. It has been previously shown that this tumour model, HOC18, is, in general, quite susceptible to chemotherapeutic agents such as CDDP, paclitaxel, doxorubicin and cyclophosphamide [33], all drugs widely used in the clinical setting. It is reasonable to speculate that tumours overexpressing cyclins and not expressing CDK

inhibitors should be more sensitive to treatment with anticancer drugs whose action is greater in cycling cells [10].

By analysing the response of these tumors to CDDP treatment and the expression of the different genes examined, no definitive conclusions could be drawn. The most responsive tumours to CDDP treatment seemed to be those expressing high levels of *BAX* and low levels of *KIP1*, *cyclin E*, *ERCC3* and *GST*π, although the limited number of samples did not allow any reliable statistical analysis.

The CDDP resistant variant Y-HOC8, a variant of P-HOC8 [14], showed a notable decrease in the expression of mRNA for *WAF1*, *GADD45* and *BAX*, three genes activated by wild-type *p53*. This decrease, however, was not due to mutations in the *p53* gene in the resistant variant, as reported in some cell systems selected for resistance to CDDP [34]. One possibility could be that, in this tumour, *p53* (if it plays any role) is present but not active, suggesting the possibility that the 'sensors' responsible may not be functional as has been reported, for example, in cells derived from Ataxia telangiectasia patients [35].

In summary, we determined the molecular characterisation of a large panel of human ovarian cancer xenografts which are of potential interest for the evaluation of specific therapeutic agents for this neoplasm. These characterised tumour models are a potentially useful system to evaluate the biochemical and biological parameters which predict the antitumour activity of new drugs, thus providing a preclinical basis for rational clinical development.

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