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Research Article

Gene expression profiles in the cellular response to a multinuclear platinum complex

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Abstract. We used cDNA arrays to monitor modulation of mRNA expression after exposure to a multinuclear platinum complex (BBR3464) in a human cervix squamous cell carcinoma cell line (A431) and in a cisplatin-resistant subline (A431/Pt) exhibiting collateral sensitivity to BBR3464. In parental A431 cells, the drug induced at least twofold up-regulation of 15 genes including cell cycle and growth regulators, tumor suppressors and signal transduction genes. In cisplatin-resistant A431/Pt cells, BBR3464 increased the expression of 15 genes such as apoptosis regulators and genes involved in the DNA damage response. Interestingly, BBR3464 induced up-regulation of anti-metastatic factors together with down-regulation of several pro-metastatic factors. Cell cycle analysis indicated a marked G2 arrest in treated A431 cells, whereas an apoptotic response was documented in A431/Pt cells. These differential patterns of transcriptional profile in sensitive and resistant cells are consistent with a role for cell cycle regulation in the response to BBR3464.

Key words. Platinum compound; sensitivity; resistance; gene expression; cDNA array.

Platinum drugs are used in the therapy of several human tumors. Despite the efficacy of platinum-based therapies, resistance often occurs [1, 2]. The cellular mechanisms of resistance to cisplatin have been shown to involve multiple alterations including (i) decreased drug accumulation [3–5], (ii) increased detoxification [6], (iii) enhanced repair of DNA damage [7, 8], (iv) mismatch repair deficiency [4, 5, 9, 10] and (v) reduced susceptibility to drug-induced apoptosis [11]. Thus, resistance to cisplatin involves increased expression of defence factors as well as altered cellular responses to the genotoxic damage. Among the recently developed platinum-containing drugs, the trinuclear platinum complex BBR3464 exhibits promising features, including lack of cross-resistance with cisplatin [12, 13]. Several lines of evidence indicate that the altered expression of a subset of genes influencing critical pathways may be relevant in determining cellular responses to cytotoxic agents [14–17]. Various alterations of regulatory genes associated with transformation may also influence cellular sensitivity to chemotherapeutic drugs. These genetic alterations affect distinct gene products including tumor suppressor genes, oncogenes, transcription factors, growth regulators, DNA repair genes and cell cycle and cell death regulators. The development of cDNA array technology has allowed the simultaneous analysis of the expression of hundreds of genes. cDNA array technology, in combination with bioinformatic tools, is expected to provide novel insights in different fields including tumor biology and anti-tumor pharmacology [18–20]. Studies performed in human tumors demonstrate the potential of a modern molecular taxonomy based on the statistical power of large phenotypic datasets, and a disease classification created on the basis of phenotypes [21–23]. This characterization defines the phenotype as that set of genes whose expression is up- or down-regulated when contrasting datasets obtained in different bio-

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logical conditions [24, 25]. Since the fate of drug-treated cells could depend on activation/repression of multiple biochemical pathways, in the present study we used cDNA arrays to monitor modulation of mRNA expression after drug exposure in two cell lines characterized by specific patterns of response to the trinuclear platinum complex BBR3464. A cDNA expression array containing genes involved in cell cycle/growth regulation, apoptosis, the DNA damage response, tumor progression, transformation and invasion was selected because the genotoxic stress was expected to modulate at least some of the represented pathways. The pair of cisplatin-sensitive (A431) and cisplatin-resistant (A431/Pt) cells was chosen for analysis of modulation of mRNA expression after BBR3464 exposure, because the A431/Pt cells exhibit collateral sensitivity to the trinuclear complex. Differential gene expression profiles were examined using commercially available cDNA arrays containing 588 cancer-related genes. Our results suggests that the diverse transcriptional profiles activated by BBR3464 in the two cell lines with different BBR3464 sensitivities may provide clues to the different cell fates after drug exposure. Defining gene expression signatures induced by anti-tumor agents may suggest novel targets for therapeutic modulation.

Materials and methods

Cell lines and growth conditions

The human cervix squamous cell carcinoma A431, and the cisplatin-resistant variant A431/Pt were used in this study [4]. Both cell lines were grown as monolayers in RPMI-1640 medium (Bio-Whittaker, Verviers, Belgium) supplemented with 10% fetal bovine serum (Invitrogen, Paisley, UK).

RNA isolation

Exponentially growing cells were seeded in 150-cm^2 flasks and 48 h later were exposed to BBR3464 for 1 h. BBR3464 was prepared as the NO_3^- salt [26] and dissolved in saline before use. After drug exposure, cell monolayers were washed and incubated in drug-free medium for 24 h. Cells were then harvested and total RNA was isolated by LiCl precipitation and phenol-chloroform extraction. After DNase treatment, RNA quality was checked by gel electrophoresis.

Labeling of cDNA probes and hybridization to cDNA arrays

The Atlas Human Cancer cDNA Expression Arrays (Clontech, BD Biosciences, Palo Alto, Calif.) were used. The filter consists of cDNAs of 588 genes relevant to cancer as duplicate spots. The complete list of cDNAs and control immobilized on the array is available at www.clontech.com. Probe synthesis and purification, hybridization and washings were performed according to the manufacturer's instructions. The radioactively labeled cDNA probes were prepared from 5 µg of total RNA in the presence of [α^{32}P]dATP (3000 Ci/mmol, 10 mCi/ml; Amersham, Little Chalfont, UK) and were purified using CHROMA SPIN-200 columns (Clontech, BD Biosciences). After overnight hybridization and washings, filters were exposed overnight and scanned at 88-µm resolution using a PhoshorImager (Molecular Dynamics, Sunnyvale, Calif.).

cDNA array data analysis

The data analysis (i.e. determination of the intensity of the signal for each spot) and evaluation (i.e. comparisons of two arrays and generation of a gene list) were performed with the AtlasImage 1.5 software. For calculating the background, the 'Default External' method was used, in which the background is set at the median intensity of the 'blank space' between the different panels of the array. With this method, the threshold level can be adjusted to a signal intensity at which a spot is considered 'real' (i.e. above the background level). Adjusting this 'Signal Threshold' permits one to filter out weak signals that are not attributable to actual gene expression or that are too weak for meaningful interpretation. By default, the 'Background Value' is used to establish the 'Signal Threshold.' The threshold is set in such a way that any gene whose 'Adjusted Intensity' (average intensity of left and right spots minus background) is at least two-fold the 'Background Value,' can be considered a genuine signal. To avoid any false-positive results and to detect significant expression changes, we used threshold values of an intensity ratio of 2.0 for up-regulation and 0.5 for down-regulation. To normalize the signal intensity between the two compared arrays, we used the 'Global Normalization' method, in which the signal values of all genes on the array are considered. In the global normalization mode, the 'Sum method' is the default method that adds the values of signals over background for all genes on the arrays to calculate the 'Normalization Coefficient.' Gene expression levels were not normalized using housekeeping genes included in the array, because under our conditions, their expression could be potentially altered by drug exposure.

Western blot analysis

Cell lysates from untreated and drug-treated cells were prepared as previously described [27]. Briefly, samples (80 µg/lane) were fractionated by SDS-polyacrylamide gel electrophoresis and blotted on nitrocellulose sheets. Blots were pre-blocked for 1 h at room temperature in phosphate-buffered saline containing 5% (w/v) dried nonfat milk. Filters were incubated overnight at 4 °C with monoclonal antibody to $p21^{WAF1}$ (NeoMarkers, Fremont, Calif.), cyclin B1 (Santa Cruz Biotechnology, Calif.) and

cyclin C (Biosource International, Camarillo, Calif.); a rabbit anti-actin antibody (Sigma, St. Louis, Mo.) was used as a control for loading. Antibodies binding to the nitrocellulose blots were detected by chemiluminescence procedures (Amersham Pharmacia Biotech Italia, Cologno Monzese, Milan, Italy).

Reverse transcriptase-polymerase chain reaction analysis

For RT-PCR analysis, 2 µg of total RNA was converted to cDNA with the Superscript First-Strand Synthesis System (Invitrogen S.R.L., San Giuliano Milanese, Italy). The cDNAs were used to amplify wee1 and cdk4 genes (wee1 primers: 5′-gcagatatttttgcgcttgc-3′/5′-taccagtgccattgctgaag-3′; cdk4 primers: 5′-ccccgaagttcttctgcagtc-3′/5′-gaaaggcagagattcgcttg-3′). In each sample, forward and reverse primers for β-actin (5′-gaaactaccttcaactccatc-3′/5′-ggcggctccatcctggcctcg-3′) were added and the amplified product was used as a control for the densitometric analysis. PCR conditions were as follows: initial denaturation at 95 °C for 9 min, followed by 30 cycles at 95 °C for 1 min, 54°C (for wee1)/56 °C (for cdk4) for 1 min, 72 °C for 1 min and finally 72 °C for 10 min.

Analysis of cell cycle perturbations

Cell cycle distribution was assessed by determining the DNA content by propidium iodide staining. For determination of propidium iodide-stained cells, exponentially growing cells were seeded in 25-cm^2 flasks and then exposed to the drug for 1 h. Twenty-four hours after treatment, floating and adherent cells were detached, washed with phosphate-buffered saline, fixed in 70% ice-cold ethanol, rehydrated in phosphate-buffered saline and stained in propidium iodide (30 µg/ml) solution containing RNase A (66 U/ml) for 30 min. The fluorescence intensity was determined by a FACScan flow cytometer equipped with an argon laser (Becton Dickinson, Mountain View, Calif.). Ten thousand cells/sample were analyzed for DNA content and cell cycle distributions were calculated with the Lysys II software (Becton Dickinson).

Apoptosis studies

Cells were seeded in 75-cm^2 flasks and after 24 h exposed to BBR3464 for 1 h. Twenty-four hours later, adherent and floating cells were harvested and apoptosis was measured by a Tdt-mediated dUTP nick end labeling (TUNEL) assay (Promega, Madison, Wis.) using flow cytometry.

Results

Study design and comparisons

The human cervix squamous cell carcinoma cell line A431 and the cisplatin-resistant subline A431/Pt were used because A431/Pt cells exhibit collateral sensitivity to the trinuclear platinum complex BBR3464 (table 1). We compared (i) untreated cisplatin-sensitive A431 cells and A431 cells exposed to BBR3464 and (ii) untreated cisplatin-resistant A431/Pt cells and A431/Pt cells exposed to BBR3464. Short-term exposure to the drug was chosen because BBR3464 exhibits a high cytotoxic potency, and a 1-h treatment is sufficient for significant interaction of platinum compounds with DNA, as documented in previous cellular pharmacology studies [5, 13]. After drug exposure, cells were incubated for 24 h in drug-free medium to allow transcriptional activation of the cell response. Drug concentrations used for comparing cDNA expression profiles corresponded to the IC_{80} (concentration inhibiting cell proliferation by 80%). Moreover, studies of drug/DNA interaction indicated that the chosen BBR3464 concentrations (10 µg/ml for A431 cells and 0.3 µg/ml for A431/Pt cells) generate a similar amount of Pt adducts into DNA (data not shown). Human cancer cDNA arrays including 588 genes were employed.

Table 1. Sensitivity of A431 and A431/Pt cells to BBR3464.

Cell line	IC_{50} (µg/ml)	IC_{80} (µg/ml)
A431	3.1 ±1.8	8.0 ± 1.8
A431/Pt	0.03±0.01	0.4 ± 0.05

Cell sensitivity to the drug was assessed by growth-inhibition assays. Cells were exposed for 1 h to BBR3464 and counted 72 h later. Values are the mean (±SD) of four independent experiments.

Drug-modulated transcripts in A431 cells

After the cDNA array hybridizations, data analysis, normalization and evaluation were performed with specific software. For the two different investigated conditions, genes that were at least two fold up- or down-regulated were listed. A typical scanned phoshorimage of one of the experiments is shown in figure 1.

In the case of A431 cells, drug treatment resulted in up-regulation of 15 of 588 genes (table 2). These genes included cell cycle and growth regulators ($p21^{WAF1}$, wee1, cyclin B1, CDC27, PLK1, CDC6-related protein), tumor suppressors (STAT1) or putative tumor suppressors (LRP1) and signal transduction genes (RhoC, RhoHP1). The up-regulation of two cell cycle inhibitors ($p21^{WAF1}$, acting mainly in G1/S and Wee1, which is a G2/M inhibitor) was indicative of a possible attempt by A431 cells to arrest the cell cycle in response to BBR3464.

We also found up-regulation of STAT1 and IRF1, two tumor suppressors cooperating in interferon-gamma (IFN-γ)-mediated cell growth inhibition [28, 29]. STAT1 negatively regulates angiogenesis, tumorigenicity and metastasis of tumor cells and its expression has been shown to be associated with an IFN-γ-dependent immune response against tumors [30]. More interesting, a role for STAT1 has been demonstrated in the induction of $p21^{WAF1}$

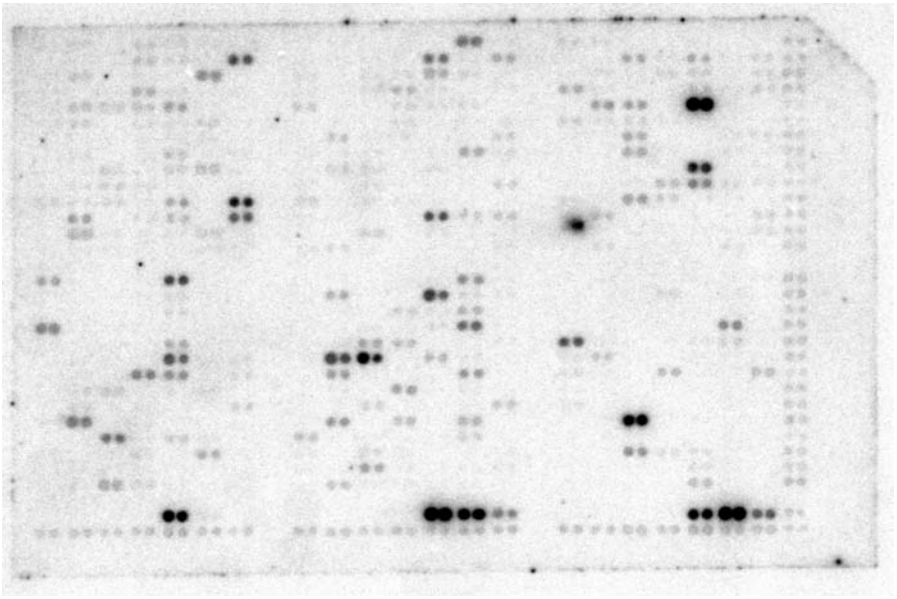

Figure 1. Typical image of human cancer cDNA expression array scanned by PhosphorImager. Array hybridization from untreated A431 cells is shown.

expression in many cell types, alternatively to p53 [31]. This evidence appears particularly relevant in our cell system, because A431 cells are characterized by a mutated and easily inactivated p53 (codon 273: C**G**T→C**A**T) [11, 32].

Moreover, the STAT1/IRF1 pathway is involved in activation of caspase-1 and -8 and in apoptosis initiated by IFN-γ in resistant tumor cells and, specifically, in A431 cells [33, 34]. The mechanism by which genotoxic stress induces IRF1, and the signaling components upstream of this anti-oncogenic transcription factor during the response to DNA damage are not yet known. However, IRF1 is up-regulated during the response to DNA damage in an ATM-dependent manner, thereby contributing to the regulation of $p21^{WAF1}$ expression [35].

Our analysis also revealed up-regulation of two members of the Rho family (RhoC, RhoHP1), which are possibly involved in the response to stress-inducing agents and in p53-independent apoptosis mediated by the generation of ceramides [36–38]. RhoC GTPase is a member of the Ras superfamily of small GTPases, whose activation leads to assembly of actin-myosin contractile filaments and focal adhesion complexes, and to a general dynamic reorganization of the cell cytoskeleton [39, 40]. Recently, several studies have shown links between RhoC and the invasive phenotype in different tumor types. However, there is evidence to suggest that the Rho proteins may be immediate-early inducible by various DNA-damaging agents, including cisplatin [41].

Another induced factor was the receptor tyrosine kinase EphA2 that is regulated by the p53 family proteins and induces apoptosis after DNA damage [42]. Additional up-regulated genes were P-cadherin, envoplakin and two membrane receptors (LRP1 and MG160).

Exposure of A431 cells to BBR3464 resulted in a significant down-regulation of only four genes, including receptors implicated in signal transduction (UFO/Axl and EPO-R oncogenes), a DNA repair protein (RAD23) and a factor belonging to the Jag2/Notch signal transduction pathway, whose constitutive activation is connected with

Table 2. Genes modulated by BBR3464 exposure in A431 cells.

Gene code	Ratio	Difference	Gene/protein name
A2j	3	49	cyclin B1
A3e	3.5	62	$p21^{WAF1}$
A3j	2.6	40	wee1
A3k	3.4	60	PLK1
A4a	2.2	29	CDC27
A4e	3.3	57	CDC6-related protein
B7l	3.03	61	STAT1
D7i	2.1	27	MG160 (GLG1)
E2n	2.1	31	LRP1
E3m	2.3	87	RhoC
E4h	2.3	92	RhoHP1
E5f	2.2	52	cadherin 3
E6k	2.8	34	EVPL
E7i	2.5	35	EphA2
F7g	2.02	35	IRF1
C5b	0.46	– 29	UFO/Axl
C5i	0.5	–139	EPO-R
C3e	0.5	– 33	RAD23
C3k	0.5	– 34	JAG2

Cisplatin-sensitive A431 cells were exposed to 10 µg/ml BBR3464 for 1 h and 24 h later were processed for RNA extraction and array hybridization.

Table 3. Genes modulated by BBR3464 exposure in A431/Pt cells.

Gene code	Ratio	Difference	Gene/protein name
A1d	2.5	48	CDK4
A2k	2.1	30	cyclin C
A5h	2.3	26	E2F1
A6i	2.07	56	MM1
B6f	2.1	22	MSH6
B7d	2.4	27	RBQ1
C1n	2.2	30	DNA topoisomerase II α
C3d	2.2	23	HHR6A
E1k	2.2	56	MMP14
E3e	2.2	109	NM23-H1
E4f	2.05	20	TC10
E5f	2.2	70	cadherin 3
E6f	2.3	25	SAP102
E7g	2.3	25	EFNB2
F1g	2.1	21	BMP1
B2g	0.5	–24	TRIP
F4g	0.40	–98	IL-1β

Cisplatin-resistant A431/Pt cells were exposed to 0.3 µg/ml BBR3464 for 1 h and 24 h later were processed for RNA extraction and array hybridization.

deregulated cell proliferation [43]. Down-regulation of receptors is consistent with the drug-induced growth inhibitory effect.

Drug-modulated transcripts in A431/Pt cells

Analysis of the gene expression profile of A431/Pt cells after BBR3464 treatment showed up-regulation of 15 genes (table 3). Among modulated genes, only P-cadherin was modulated in both A431 and A431/Pt cells. Interestingly, up-regulation of some genes implicated in apoptosis induction was found. In particular, we observed over-expression of E2F1 that, in addition to its established role in proliferation, has also been implicated in the induction of p53-dependent or -independent (p73-mediated) apoptosis following DNA damage, by activation of Apaf-1 and caspase 7 [44–46]. Recent evidence suggests that over-expression of E2F1 promotes apoptosis by inhibiting NF-κB activity and by reducing TRAF2 protein levels [47, 48]. Moreover, a plausible explanation for the tumor suppressor activity of E2F1 may lie in its potential capability to repress transcription of several genes [49, 50]. Treatment resulted in down-regulation of only two transcripts [TRAF-interacting protein and interleukin (IL)-1β)].

Additional drug-modulated transcripts

A more sophisticated analysis of the expression profiles including genes modulated to an extent close to that set with the software was undertaken. The analysis revealed additional putative interesting genes in the two cell lines (tables 4, 5) and led to a slight increase in the number of genes modulated in both systems (globally, eight transcripts similarly modulated in both cell lines; table 6). This analysis revealed two 'common' pathways shared by A431 and A431/Pt cells. In particular, we observed up-regulation of anti-metastatic factors (Nm23-H2in A431 cells, Nm23-H1and SAP102 in A431/Pt cells and CD9 in both cell lines) together with down-regulation of several pro-metastatic factors (UFO/Axl, EpoR, JAG2and VEGF in A431 cells and IL-1β in A431/Pt cells). Together, BBR3464 treatment appears to produce specific changes

Table 4. Genes slightly modulated by BBR3464 exposure in A431 cells.

Gene code	Ratio	Difference	Gene/protein name
A4d	1.8	38	CDC 37 homolog
A6i	1.8	35	MM1
D5f	1.8	147	CD9
E1k	1.8	27	MMP14
E3f	1.8	203	NM23-H2
E4e	1.8	30	RAC1
E6g	1.9	79	EB1
F1e	0.6	–67	VEGF

Cisplatin-sensitive A431 cells were exposed to 10 µg/ml BBR3464 for 1 h and 24 h later were processed for RNA extraction and array hybridization.

Table 5. Genes slightly modulated by BBR3464 exposure in A431/Pt cells.

Gene code	Ratio	Difference	Gene/protein name
A2j	1.9	48	cyclin B1
C7d	1.9	28	p68 TRK-T3
D5f	1.9	142	CD9
D5k	1.8	28	ninjurin 1
E4h	1.9	54	Rho-HP1
E5d	1.8	24	PAK γ
E6d	1.9	194	plakoglobin
E6g	1.9	111	EB1
E6j	1.8	79	desmoplakin I and II
E6k	1.8	26	EVPL

Cisplatin-resistant A431/Pt cells were exposed to 0.3 µg/ml BBR3464 for 1 h and 24 h later were processed for RNA extraction and array hybridization.

Table 6. Genes modulated by BBR3464 exposure in both A431 and A431/Pt cells.

Gene name	Ratio A431	Ratio A431/Pt
Cyclin B1	3	1.9
RhoHP1	2.3	1.9
Cadherin 3	2.2	2.2
EVPL	2.8	1.8
MM1	1.8	2.1
MMP14	1.8	2.2
CD9	1.8	1.9
EB1	1.9	1.9

Cisplatin-sensitive A431 and -resistant A431/Pt cells were exposed to BBR3464 for 1 h and 24 h later were processed for RNA extraction and array hybridization.

resulting in anti-metastatic behavior in both systems. Induction of several factors belonging to a family of versatile cytolinker proteins (plakins) was also found. In fact, BBR3464 exposure induced expression of envoplakin in both cell lines and of plakoglobin and desmoplakin in A431/Pt cells. By connecting cytoskeletal elements to each other and to junctional complexes, the plakin family of cytolinkers plays a crucial role in orchestrating cellular development and maintaining tissue integrity [51]. Although the possible role of plakins in the platinum drug response remains to be defined, their presence in organelles with known signaling functions as well as their interactions with signaling proteins and their associations with the actin cytoskeleton suggest that plakins are likely involved in regulating diverse cellular events such as cell growth, programmed cell death and cell migration.

Validation of selected changes

To validate the changes observed by cDNA array analysis, Western blot and RT-PCR were used (fig. 2). Expression levels of $p21^{WAF1}$, cyclin B1 and cyclin C were analyzed by Western blotting, while RT-PCR was employed to estimate wee1 and cdk4 mRNA levels. Differential $p21^{WAF1}$ expression was observed when comparing A431 and A431/Pt cells. BBR3464 exposure increased the $p21^{WAF1}$ protein level only in the sensitive cells, whereas no modulation was found in the A431/Pt cell line. Cyclin B1 was up-regulated after BBR3464 exposure in both sensitive and resistant cell lines. BBR3464 increased cyclin C only in the resistant subline. In agreement with cDNA array analysis, BBR3464 exposure induced up- regulation of wee1 mRNA in A431 cells (1.5-fold) and cdk4 mRNA in A431/Pt cells (1.8-fold).

Cell cycle perturbations after exposure to BBR3464

Treatment with platinum compounds is known to induce an arrest in the G2 phase of the cell cycle independently of p53 status [52]. Since a protective role against DNA damage has been assigned to the G2 checkpoint, we compared the cell cycle distribution in control and BBR3464-treated cells. Cisplatin-sensitive and -resistant cells were exposed for 1 h to drug concentrations coresponding to the IC_{80}, which inhibit cell growth to the same extent and generate similar levels of DNA adducts. Cell cycle perturbations were examined 24 h later. At the tested concentrations, BBR3464 caused an accumulation of both A431 and A431/Pt cells in the G2/M phase (Table 7). However, the G2/M arrest was more evident in A431 than in A431/Pt cells, as shown by comparison between the percentage of G2/M-accumulated cells in untreated and BBR3464-treated cells. Moreover, a higher fraction of sub-G1 cells, representative of apoptotic cells, was found after BBR3464 exposure of A431/Pt cells.

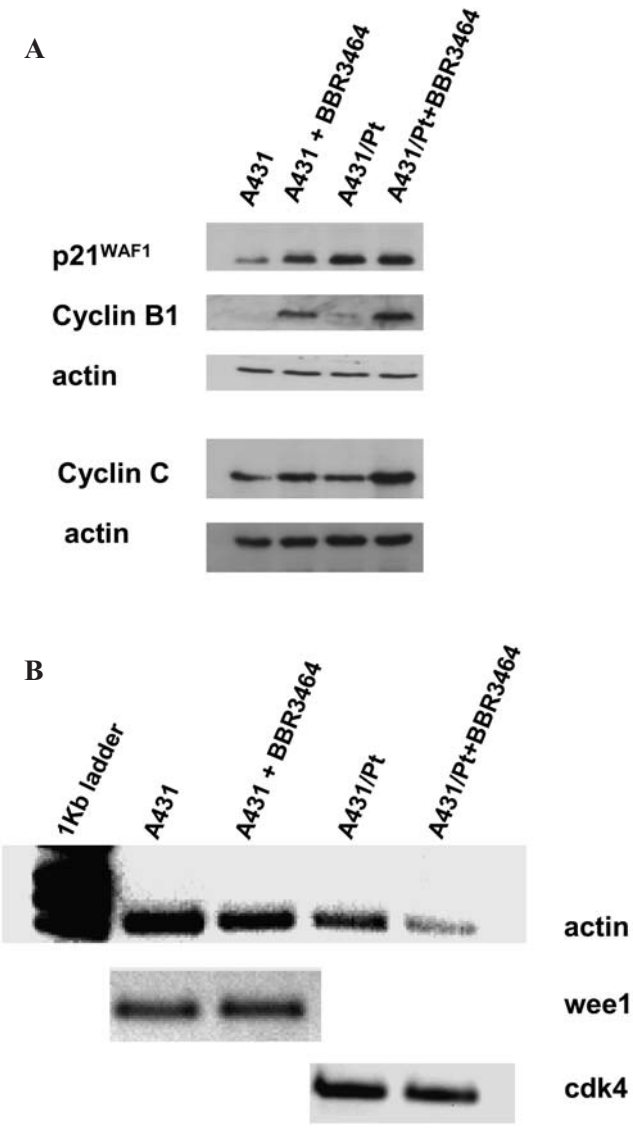

Figure 2. Western blot and RT-PCR analysis. (*A*) Expression levels of $p21^{WAF1}$, cyclin B1 and cyclin C. Protein levels were analyzed by Western blot and normalized with respect to actin. (*B*) Expression levels of wee1 and cdk4. mRNA levels were analyzed by RT-PCR and were normalized with respect to actin.

Table 7. Cytofluorimetric analysis of BBR3464-induced cell cycle perturbations in A431 and A431/Pt.

	Sub-G1	G1	S	G2/M
A431				
Control	1.9	53.1	6.5	38.5
BBR3464	2.6	9.6	2.8	85.0
A431/Pt				
Control	1.2	51.0	10.3	37.5
BBR3464	9.5	21.6	4.0	64.9

Exponentially growing cells were treated with BBR3464 for 1 h; 24 h later, cells were stained with propidium iodide. Fluorescent intensity was determined by FACScan. The percentage of cells in different cell cycle phases is shown.

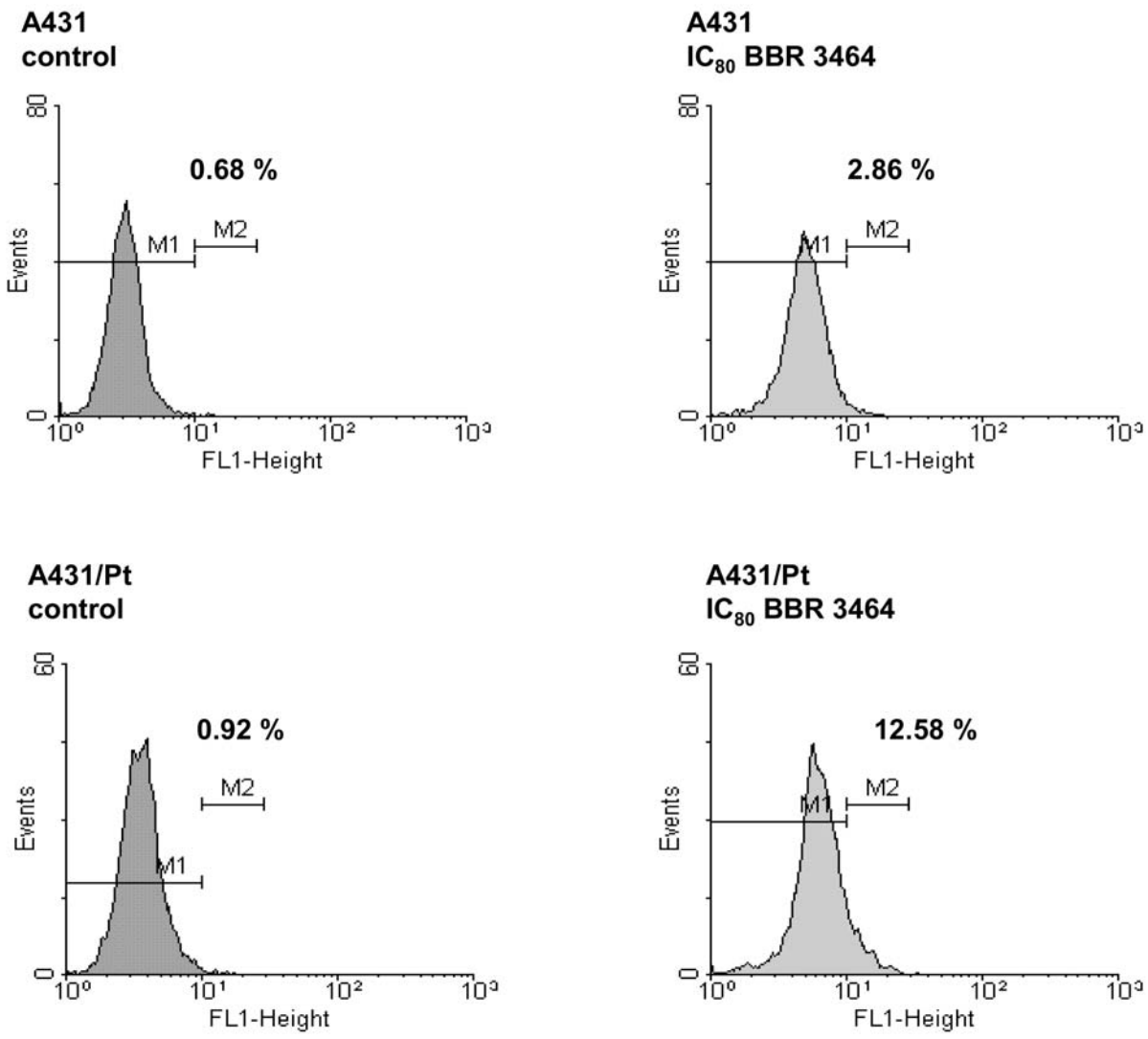

Figure 3. BBR3464-induced apoptosis in A431 and A431/Pt. Exponentially growing cells were treated with BBR3464 for 1 h; 24 h later cells were harvested and apoptosis was measured by TUNEL assay. M1, live cells; M2, apoptotic cells.

Drug-induced apoptosis

The relationship between cell sensitivity and apoptosis was examined in A431 and A431/Pt cells, to determine whether the increased sensitivity to BBR3464 in the cisplatin-resistant subline resulted in apoptotic cell death. Although previous studies suggested that apoptosis was not the major mode of cell death in response to cisplatin in these cells [4], flow cytometric analysis of apoptotic cells by TUNEL assay indicated that the extent of drug-induced apoptosis was higher (five-fold) in A431/Pt than in A431 cells (fig. 3).

Discussion

In this study, we combined a molecular approach with conventional pharmacology tools to define the mechanisms of the cellular response to BBR3464. To dissect out the contribution of multiple factors, we examined the gene expression profiles of the two isogenic cell systems using cDNA arrays containing 588 genes. Although in both cell lines most of the modulated transcripts belong to the subgroup of genes involved in regulation of the cell cycle or apoptosis, the cellular response activated by drug treatment appears to differ in the parental A431 and the A431/Pt variant. In the A431 cell line, up-regulation of $p21^{WAF1}$ mRNA was found, whereas such a change was not observed in the cisplatin-resistant variant. An opposite response was observed after cisplatin exposure: up-regulation of $p21^{WAF1}$ was detected only in the cisplatin-resistant subline (data not shown). This finding is consistent with a protective role of $p21^{WAF1}$ in response to DNA damage. However, the opposite behavior of the two cell lines implies the involvement of different signaling pathways ac-

tivated by different types of DNA lesions. In addition to the well known role of $p21^{WAF1}$ in G1/S arrest, recent evidence suggests that it could also participate in G2 arrest after DNA damage, because it inhibits Cdc2 activity by both p53-dependent and -independent mechanisms [53, 54]. A number of transcripts also involved in regulating G2/M progression including cyclin B1, wee1, cdc27 and PLK1 were up-regulated by treatment in A431 cells, whereas only cyclin B1 levels were increased in A431/Pt cells. Conclusive interpretation of such findings is not possible because the final activity of several factors involved in cell cycle regulation is affected by post-translational modifications (e.g. phosphorylation). The gene expression profile of treated A431 cells, which are less sensitive to BBR3464, indicates the modulation of several factors which could favor inhibition of progression through mitosis (wee1, which inhibits the CDC2/cyclinB1 complex; cdc27 and PLK1, implicated in cyclin B1 degradation) and likely activation of repair processes. In this regard, $p21^{WAF1}$ could play a role by interacting with PCNA which is implicated in repair processes [55]. Despite up-regulation of cyclin B1 in A431 cells, the levels of factors promoting its degradation (cdc27 and PLK1) or inactivating the CDC2/cyclin B1 complex (i.e. wee1 and $p21^{WAF1}$) were increased. These findings suggest that G2 arrest could be more pronounced in A431 cells than in A431/Pt cells in which cdc27 and PLK1 were not repressed. Such an interpretation is supported by the results obtained from cytofluorimetric analysis of drug-induced cell cycle perturbations (table 7).

In the drug-treated A431/Pt cell line, hypersensitive to the effect of BBR3464, we found up-regulation of factors implicated in apoptosis induction including E2F1, cyclin C, MSH6 and DNA topoisomerase II α [44, 56]. This specific expression profile is associated with an increased susceptibility to drug-induced apoptosis in A431/Pt as compared to A431 (fig. 3).

Regarding modulation of genes involved in the metastatic process, BBR3464 produced up-regulation of anti-metastatic factors (Nm23-H2 in A431 cells, Nm23-H1 and SAP102 in A431/Pt cells, and CD9 in both cell lines) as well as down-regulation of pro-metastatic factors in both cell systems (UFO/Axl, EpoR, JAG2 and VEGF in A431 cells and IL-1β in A431/Pt cells). The specific transcriptional response in the two cell lines at concentrations producing equal levels of DNA platination is consistent with their relative sensitivity. These results support the potential value of this molecular analysis to identify those genes which critically determine chemosensitivity and are potential targets for pharmacological intervention. The identification of the pattern of gene expression and drug-induced modulation in cell systems could have relevant implications in an attempt to provide a rationale for optimization of tumor treatment based on the molecular features.

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