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ORIGINAL ARTICLE

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Screening of an annotated compound library for drug activity in a resistant myeloma cell line

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Abstract *Purpose*: Resistance to anticancer drugs is a major problem in chemotherapy. In order to identify drugs with selective cytotoxic activity in drug-resistant cancer cells, the annotated compound library LOPAC$_{1280}$, containing compounds from 56 pharmacological classes, was screened in the myeloma cell line RPMI 8226 and its doxorubicin-resistant subline 8226/Dox40. *Methods*: Cell survival was measured by the Fluorometric Microculture Cytotoxicity Assay. *Results*: Selective cytotoxic activity in 8226/Dox40 was obtained for 33 compounds, with the most pronounced difference observed for the glucocorticoids. A microarray analysis of the cells showed a difference in mRNA-expression for the glucocorticoid receptor suggesting potential mechanisms for the difference in glucocorticoid sensitivity. In the presence of the glucocorticoid-receptor antagonist RU486, the sensitivity to the glucocorticoids was reduced and a similar effect level in RPMI 8226 and 8226/Dox40 was achieved. *Conclusion*: In conclusion, screening of mechanistically annotated compounds on drug-resistant cancer cells can identify compounds with selective activity and provide a basis for the development of novel treatments of drug-resistant malignancies.

Keywords Anticancer drug resistance · Drug screening · Annotated compound library · Glucocorticoids · Gene expression

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Introduction

Lack of significant antitumour effect of cytotoxic drugs is a frequently observed phenomenon in chemotherapy of malignant tumours [22]. Tumours may be intrinsically resistant or a resistance phenotype can be acquired during the course of chemotherapy [18, 22]. Cellular resistance has been studied extensively in cell line models with acquired resistance from exposure to increasing concentrations of an anticancer drug [10]. Mechanisms for drug resistance derived from such models are numerous and include alterations of drug transport, drug metabolism, DNA synthesis and repair, cell survival and apoptosis [10, 18, 24]. Thus, therapeutic strategies to overcome drug resistance are an urgent requirement.

The development of novel molecular technologies, such as expression microarrays has made it easier to identify genes involved in cellular drug resistance. Integration of gene expression and drug activity data sets for cancer cells can identify relationships between individual genes and sensitivity or resistance to specific drugs [26, 28]. A complementary approach for biological mechanism profiling using small organic molecules was recently introduced [27]. The approach is based on cell-based high throughput screening (HTS) of an annotated compound library representing a large-scale collection of compounds with diverse and experimentally validated biological mechanisms and it was capable of identifying novel lead compounds with cancer phenotypic selectivity as well as generating hypotheses regarding the underlying biological mechanism [27].

In the present study, an annotated compound library consisting of 1,266 well-characterized compounds was screened in sensitive and drug-resistant cancer cell lines to explore the possibility to find compounds with

selective cytotoxic activity against resistant phenotypes. Concurrently, the cells were profiled with CodeLink® Bio-arrays to detect differences in gene expression between the sensitive and resistant cell lines.

Materials and methods

Cell culture

The myeloma cell lines RPMI 8226, 8226/Dox40 and 8226/LR5 were kind gifts from WS Dalton (H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA). The leukaemia cell lines CCRF-CEM and CEM/VM-1 were kind gifts from WT Beck (University of Illinois at Chicago, Chicago, USA). The small cell lung carcinoma cell lines NCI-H69 and H69AR were from the American Type Culture Collection (Manassas, VA, USA). The doxorubicin-resistant 8226/Dox40 over expresses *P*-glycoprotein/MDR1/ABCB1 [3]. The resistance to melphalan in 8226/LR5 is proposed to be associated with increased levels of glutathione and genes involved in cell cycle and repair [13]*. The doxorubicin-resistant H69AR over expresses MRP1/ABCC1 [2, 20, 29]. The resistance to teniposide in CEM/VM-1 is proposed to be topoisomerase II associated [5, 6, 19]. The cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin (all from Sigma-Aldrich Co, St Louis, MO, USA) at 37°C in humidified air containing 5% CO_2. The resistant cell lines were tested regularly for maintained resistance to the selected drugs. Growth and morphology were monitored on a weekly basis.

Preparation of compounds

The LOPAC library (Sigma-Aldrich) consists of 1,266 compounds from 56 pharmacological classes (Table 1). The compounds were dissolved in dimethyl sulphoxide (DMSO) and were further diluted with phosphate buffered saline (PBS), transferred to 384-well microtiter plates (Nunc, Roskilde, Denmark) using the Biomek 2000 pipetting station (Beckman Coulter Inc, Fullerton, CA, USA) and screened at a final concentration of 10 μM. Dose-response plates containing maprotiline, salbutamol, SR 59230A, eseroline, 4′-chloro-3alpha-(diphenylmethoxy) tropane, dihydrexidine, R(-)-2,10,11-trihydroxyaporphine, indatraline, SKF 83959, thiothixene, thioridazine, spermidine, spermine, beclomethasone, betamethasone, budesonide, dexamethasone, spironolactone, triamcinolone, U-73122, cortisone, hydrocortisone, progesterone and doxorubicin were prepared with 5 μl drug solution of ten times the desired drug concentration added to duplicate wells in tenfold serial dilution, starting from 100 μM. The drugs were dissolved in DMSO, ethanol or sterile water to a stock solution of 10 mM and further diluted with sterile water or PBS. All drugs were from Sigma-Aldrich except doxorubicin (obtained from Pfizer AB, Täby, Sweden). The plates were stored in −70°C until further use.

Measurement of cell survival

The Fluorometric Microculture Cytotoxicity Assay (FMCA) described in detail previously [17] was performed to measure cell survival. The method is based on measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA) to fluorescein by cells with intact plasma membranes. Cells were seeded in the drug-prepared 384-well plates at a density of 2,500–5,000 cells per well using the pipetting robot Precision 2000 (Bio-Tek Instruments Inc., Winooski, VT, USA). Two to three columns without drugs served as controls and one column with medium only served as blank. The plates were incubated at 37°C for 72 h and then transferred to an integrated SAGIAN™ Core System for HTS (Beckman Coulter Inc, Fullerton, CA, USA) consisting of an Optimized Robot for Chemical Analysis (ORCA; Beckman Coulter) with CO_2 incubator (Cytomat 2C, Kendro, Sollentuna, Sweden), dispenser module (Multidrop 384, Titertek, Huntsville, AL, USA), washer module (ELx 405, Bio-Tek Instruments Inc.), delidding station, plate hotels, barcode reader (Beckman Coulter), liquid handler (Biomek 2000, Beckman Coulter) and a multipurpose reader (FLUOstar Optima, BMG Labtech

Table 1 Pharmacological mechanisms in the LOPAC library

Class	Number of drugs	Class	Number of drugs
Adenosine	59	Histamine	31
Adrenoreceptor	103	Hormone	33
Angiogenesis	1	Imidazoline	11
Antibiotic	29	Immune system	11
Anticonvulsant	12	Intracellular calcium	7
Apoptosis	11	Ion pump	16
Benzodiazepine	7	K^+ Channel	17
Biochemistry	46	Leukotriene	10
Ca^{2+} Channel	17	Lipid	9
Calcium signalling	1	Lipid signalling	2
Cannabinoid	6	Melatonin	8
Cell cycle	15	Multi-drug resistance	12
Cell stress	20	Na^+ channel	17
Cholecystokinin	3	Neurotransmission	45
Cholinergic	77	Nitric oxide	37
Cl^- Channel	3	Nootropic	3
Cyclic nucleotides	31	Opioid	27
Cytokines and growth Factors	1	P2 receptor	14
Cytoskeleton and ECM	10	Phosphorylation	93
DNA	9	Prostaglandin	24
DNA metabolism	14	Prostanoids	1
DNA repair	3	Serotonin	83
Dopamine	113	Somatostatin	2
G protein	4	Sphingolipid	4
GABA	41	Tachykinin	5
Gene regulation	1	Thromboxane	2
Glutamate	88	Transcription	12
Glycine	2	Vanilloid	5

GmbH, Offenburg, Germany). Medium and drugs were aspirated, the cells were washed twice with PBS, 50 µl of physiological buffer and 1 µl of 0.5 mg/ml FDA were added and after 50–70 min of incubation, the fluorescence, which is proportional to the number of living cells, was measured at 485/520 nm in the FLUOstar Optima. Cell survival is presented as Survival Index (SI) defined as fluorescence in test wells in percent of control wells, with blank values subtracted. Quality criteria for a successful assay included a mean coefficient of variation of less than 30% in the control and a fluorescence signal in control wells of more than five times the blank.

In the inhibition experiments 10 µM of the glucocorticoid- and progesterone-receptor antagonist RU 486 (Sigma-Aldrich) was added to the cell suspension of RPMI 8226 and 8226/Dox40 before seeding in the drug-prepared plates. A control plate with drugs and cell suspension only was analysed simultaneously.

Data analysis

An Accord HTS database (Accelrys Inc, San Diego, CA, USA) and Small Laboratory Information and Management System (SLIMS: [15]) were used for screening data management and analysis. Raw data files were loaded into the SLIMS software which calculates percent inhibition according to the formula: Percent inhibition = 100 × (test well-blank/control-blank) −1. Similarity scores for compounds were calculated based on a structural fingerprint consisting of binary vectors representing structures located within the compound and which are automatically computed for each compound loaded into the program. Scores range from 0 to 1 and scores close to zero indicate structural similarity [15]. A self-organizing map (SOM) mapping the structural space of the compound library was performed by SLIMS using default settings.

Statistical analysis for dose-response experiments was performed using GraphPadPrism (GraphPad Software, Inc. San Diego, CA, USA). Data was processed using non-linear regression and a standard sigmoidal dose-response model to obtain logIC_{50}-values (inhibitory concentration 50%). Data are presented as means ± Standard Errors from three to nine independent experiments. The unpaired Student's *t* test was used to compare the IC_{50}-values within the cell line pairs and a *P*-value of < 0.05 was considered to reflect a significant difference between the activity in the parental and resistant cell lines.

RNA extraction

Total RNA was extracted from each cell line starting from 10^7 cells, using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The purity of the RNA was ensured by measuring the optical density at 260 and 280 nm. The integrity of the RNA was controlled by capillary electrophoresis using a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA). Only pure RNA (OD 260/280 > 1.8) without any sign of degradation was used in the subsequent experiments.

Microarray expression analysis

RNA samples were processed and used for CodeLink Bioarray (GE Healthcare, Piscataway, NJ, USA) hybridization as previously described [25]. For each CodeLink Bioarray, double-stranded cDNA and subsequent cRNA was synthesized from 2 µg of total RNA from each cell line using the CodeLink Expression Assay Kit (GE Healthcare) according to the manufacturer's instructions. In brief, biotin 11-UTP (PerkinElmer) was used in the in vitro transcription. The double-stranded cDNA was purified with the use of a QIAquick purification kit (Qiagen, Hilden, Germany). The labelled cRNA was purified using an RNeasy mini kit (Qiagen). Expression arrays used were CodeLink UniSet Human 20K Bioarray (GE Healthcare) containing a collection of 20,289 target probes. Bioarrays were stained with Cy5 streptavadin (GE Healthcare) and scanned using a GenePix 4000B scanner (Axon Instruments, Union City, CA, USA). Scanned image files were analysed with the use of CodeLink image and data analysis v4.0 software (GE Healthcare), which produced both raw and normalized hybridization signal intensities for each spot on the arrays. Normalized signal intensities were used in this study.

Quantitative real-time PCR (qRT-PCR) validation of microarray data

Reverse transcription of 2 µg total RNA was performed with Omniscript RT kit (Qiagen) in a volume of 20 µl using random hexamers and RNAseout (Invitrogen) according to the protocol of the manufacturer. TaqMan primers and probes for NR3C1 (Assay ID Hs00746410_s1), ACTB (product# 4326315E) and GAPD (product# 4326317E) were ordered as Assay on demand (Applied Biosystems, Foster City, CA, USA). Amplifications were performed in 25 µl reactions using TaqMan Universal PCR MasterMix (Applied Biosystems). All PCR reactions were performed in quadruplicates on ABI PRISM 7000 Sequence Detector (Applied Biosystems) with the following thermo cycling conditions: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The data was analysed and converted into threshold cycle (C_t) values with the use of the ABI Prism 7000 software system (Applied Biosystems). The C_t values were then translated into relative cDNA copy numbers by comparison to serial dilutions. The expression levels of NR3C1 were normalized to the expression of ACTB and GAPD.

Results

When screening the annotated library at 10 µM, the number of compounds with a survival index of less than

50% was 71 for RPMI 8226 and 81 for 8226/Dox40 (not shown). Thirty-three of the compounds had lower SI-values in 8226/Dox40 than in RPMI 8226 and represented 12 of the mechanistic classes, mainly dopaminergic, adrenergic and glucocorticoid compounds (Table 2). A SOM was generated by mapping the structural space of the compound library and although the selectively active compounds tended to span this representation of chemical descriptor space, a distinct cluster was observed containing many steroid compounds (Fig. 1). A plot of drug activity against the structural similarity score to the glucocorticoid betamethasone revealed a group of structurally similar compounds (Fig. 2).

To assess the sensitivity of the hit compounds, dose-response experiments were performed. The curves for RPMI 8226 and 8226/Dox40 with and without the glucocorticoid-receptor antagonist RU486 are displayed in Fig. 3. The activity of the compounds was determined by their IC_{50}-values which are summarized in Table 3. To elucidate if there was a real difference in sensitivity to the glucocorticoids between RPMI 8226 and 8226/Dox40, two-tailed unpaired Student's *t* tests were performed comparing the IC_{50}-values. The 8226/Dox40 cell line was significantly more sensitive to all tested glucocorticoids except for hydrocortisone (Students *t* test, $P < 0.01$ to < 0.05, $n = 6$–9, Table 3). The sensitivity to doxorubicin was also studied as a control and RPMI 8226 was more sensitive than the doxorubicin-resistant 8226/Dox40 cells ($P < 0.0001$). A resistance factor (IC_{50} in the parental divided with IC_{50} in the resistant cell line) was also calculated (Table 3). The largest difference was observed for dexamethasone where 8226/Dox40 was 30.3 times more sensitive than RPMI 8226, followed by betamethasone (25.6), budesonide (13.2), beclometha-

Fig. 1 Self-organizing map generated by SLIMS. The map shows the structure-space location of the compounds in the LOPAC library. Each node contains a collection of structurally similar compounds and each neighbouring node is more similar than non-neighbouring nodes. The hits are shown in crosshatched nodes. The *red square* indicates the location of the hit glucocorticoids

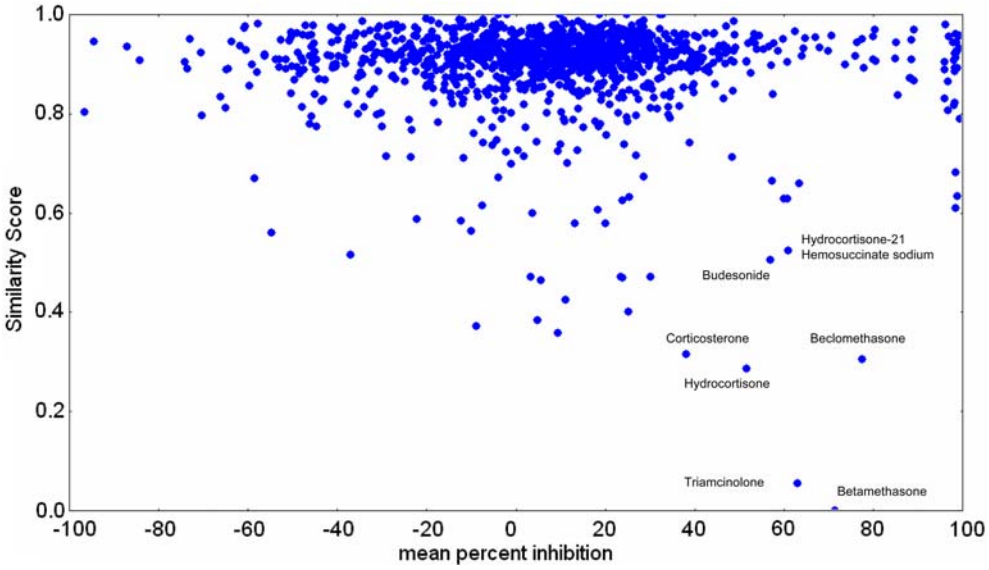

Fig. 2 Analysis of drug activity of the compounds versus structural similarity to betamethasone. Drug activity is presented as mean per cent inhibition in 8226/Dox40 cells

sone (8.7), triamcinolone (3.1) and hydrocortisone (2.5). RPMI 8226 was 100 times more sensitive to doxorubicin than 8226/Dox40.

To investigate if there were other resistant cell lines with increased glucocorticoid sensitivity, the compounds were also tested on the melphalan-resistant 8226/LR5, the leukaemia cell lines CCRF-CEM and CEM/VM-1, and the small cell lung cancer lines NCI-H69 and H69AR. For 8226/LR5, there was a statistically significant difference in sensitivity to budesonide ($P < 0.05$, $n = 3$) compared to the parental cell line and the teniposide-resistant CEM/VM-1 was more sensitive to all the tested glucocorticoids except hydrocortisone compared to the parental CCRF-CEM ($P < 0.01$ to $P < 0.05$, $n = 3$, Table 3). In contrast, no statistically significant differences in glucocorticoid sensitivity was obtained for H69 and its doxorubicin-resistant sub line H69AR (Table 3). For the dopaminergic and adrenergic compounds, no difference in IC_{50}-values was obtained among the pairs of parental and resistant cell lines (not shown).

To find differences in gene expression among the cell line pairs, microarray analysis was performed. Table 4 displays the fold changes in expression between mRNA from 8226/Dox40 compared to mRNA from RPMI 8226 for the glucocorticoid receptor and for co-activators and co-repressors present in the arrays. The expression of the glucocorticoid receptor (NR3C1) was 2.45-fold higher in 8226/Dox40 compared to RPMI 8226 (Table 4). The results were verified with qRT-PCR (Fig. 4). The other genes with difference in expression were: nuclear receptor co-activator 6 interacting protein (NCOA6IP) which showed a 2.4-fold higher expression, nuclear receptor interacting protein 1 (NRIP1/RIP140) which showed a more than tenfold higher expression and nuclear receptor binding protein (NRBP) which showed a more than twofold lower expression in 8226/Dox40.

In the presence of the glucocorticoid-receptor antagonist RU486, the sensitivity to beclomethasone, betamethasone, budesonide, dexamethasone, triamcinolone and hydrocortisone was reduced and there was no difference in IC_{50}-values between RPMI 8226 and 8226/Dox40 (Table 3).

Discussion

In the screening, several classes of compounds were found to be more active in the doxorubicin-resistant 8226/Dox40 cells, including dopaminergic, adrenergic and glucocorticoid compounds. The adrenergic and dopamine-active compounds consisted of different receptor agonists and antagonists without clear relationships to chemical structure. When testing them in concentration-response experiments, there was no difference in IC_{50}-values between the cell lines. On the contrary, the resistant cell lines 8226/Dox40 and CEM/VM-1 were more sensitive than their parental cell lines to several of the glucocorticoids. Collateral sensitivity to glucocorticoids was observed when the 8226/Dox40 cell line was developed [3], and in a study examining mechanisms of drug resistance during selection with mitoxantrone in RPMI 8226, 8226/Dox6 was found to be more sensitive to dexamethasone than its parental cell line [12], but the underlying mechanisms has not been defined. These in vitro observations show some correspondence in the clinic where patients with refractory or relapsed myeloma may respond to methylprednisolone alone [8].

The induction of glucocorticoid hypersensitivity may not be limited to doxorubicin as a selecting agent since the teniposide-resistant leukaemia cell line CEM/VM-1 also was more sensitive to the glucocorticoids compared to its parental cell line CCRF-CEM. However, there was no difference in sensitivity to any of the glucocorticoids in the lung cancer cell lines H69 and H69AR, even though the latter is expressing the multidrug-resistance protein and has been selected for doxorubicin-resistance.

Table 2 Compounds with a lower SI % at 10 μM in 8226/Dox40 compared to RPMI 8226

Name	Class	Action	Selectivity	SI % 8226/Dox40	SI % RPMI 8226
Bromoacetyl alprenolol menthane	Adrenoceptor	Antagonist	Beta	12	71
Maprotiline hydrochloride	Adrenoceptor	Inhibitor	Reuptake	45	105
Salbutamol	Adrenoceptor	Agonist	Beta2	50	92
SR 59230A oxalate	Adrenoceptor	Antagonist	Beta3	47	64
Ellipticine	Cell Cycle	Inhibitor	CYP1A1/TopoII	39	53
(-)-Eseroline fumarate	Cholinergic	Inhibitor	Cholinesterase	16	86
(±)-Chloro-APB hydrobromide	Dopamine	Agonist	D1	48	108
4′-Chloro-3-alpha-(diphenylmethoxy) tropane hydrochloride	Dopamine	Blocker	Reuptake	23	78
Dihydrexidine hydrochloride	Dopamine	Agonist	D1	3	51
R(-)-2,10,11-Trihydroxyaporphine hybrobromide	Dopamine	Agonist	D2	31	91
R(-)-2,10,11-Trihydroxy-N-propylnoraporphine hydrobromide	Dopamine	Agonist	D2	34	88
Indatraline hydrochloride	Dopamine	Inhibitor	Reuptake	20	60
L-741,626	Dopamine	Antagonist	D2	43	94
3-Phenylpropargylamine hydrochloride	Dopamine	Inhibitor	Dopamine beta-hydroxylase	4	92
SKF 83959 hydrobromide	Dopamine	Agonist	D1	45	94
SKF 89626	Dopamine	Agonist	D1	50	111
Thiothixene hydrochloride	Dopamine	Antagonist	D1/D2	35	81
Thioridazine hydrochloride	Dopamine	Antagonist	D1/D2	31	93
Spermidine trihydrochloride	Glutamate	Ligand	NMDA-Polyamine	23	95
Spermine tetrahydrochloride	Glutamate	Antagonist	NMDA-Polyamine	14	73
3-(1H-Imidazol-4-yl)propyl di(p-fluorophenyl)methyl ether hydrochloride	Histamine	Antagonist	H3	48	78
Beclomethasone	Hormone	Agonist	Glucocorticoid	23	84
Betamethasone	Hormone	Agonist	Glucocorticoid	30	68
Budesonide	Hormone	Agonist	Cortisol	45	56
Hydrocortisone 21-hemisuccinate sodium	Hormone	Agonist	Cortisol	41	92
Spironolactone	Hormone	Antagonist	Mineralocorticoid	42	102
Triamcinolone	Hormone	Agonist	Glucocorticoid	36	56
U-73122	Lipid	Inhibitor	PLC, A2	2	52
2-Chloroadenosine triphosphate tetrasodium	P2 Receptor	Agonist	P2Y	31	111
1-(5-Isoquinolinylsulphonyl)-2-methylpiperazine dihydrochloride	Phosphorylation	Inhibitor	PKA/PKC	46	119
Fluoxetine hydrochloride	Serotonin	Inhibitor	Reuptake	41	59
6-Nitroso-1,2-benzopyrone	Transcription	Inhibitor	PARP	41	57
13-cis-retinoic acid	Transcription	Regulator	RAR-alpha, beta	39	63

The difference in sensitivity can be related to the different expression of response elements in the cell type of origin where glucocorticoids are known to induce apoptosis in haematological malignancies but not in solid tumours [7, 21].

In order to find differences in gene expression among the cell line pairs, a microarray analysis was performed. We detected 2.5-fold higher levels of NR3C1 (glucocorticoid receptor) mRNA in 8226/Dox40 compared to RPMI 8226, which was verified by qRT-PCR. The level of glucocorticoid-receptor mRNA has previously been studied in RPMI 8226 and several doxorubicin-resistant cell lines, where the levels initially were low in the resistant lines and increased in the presence of dexamethasone in 8226/Dox40 [4]. Chauhan et al. [1] investigated the gene expression of a pair of dexamethasone (Dex) sensitive/resistant cell lines, where they found a decrease in glucocorticoid-receptor expression in the Dex-resistant cell line and they suggest that downregulation of GR may confer Dex-resistance in multiple myeloma cells. In an earlier study, the mRNA levels of glucocorticoid receptor and the sensitivity to glucocorticoids in RPMI 8226 were compared to other myeloma cell lines, and the authors concluded that the quantity and affinity of glucocorticoid receptor in the cells were not predictive for the response to dexamethasone and suggested post-receptor mechanisms to be more important for the glucocorticoid effect [9]. When activated, the glucocorticoid receptor moves to the nucleus, binds to DNA and initiates transcription. Depending on the environment, different genes are switched on and off and cross-talk

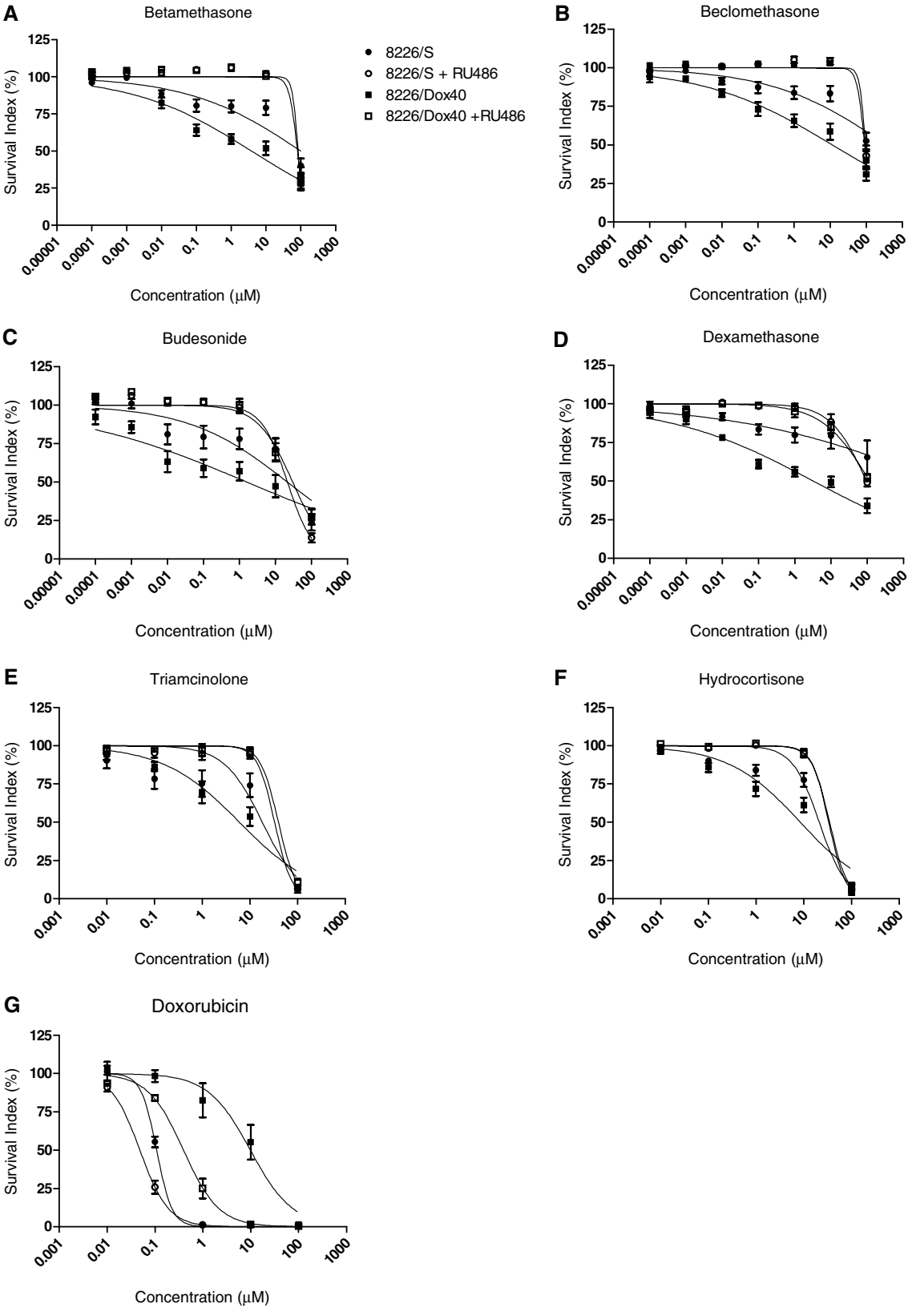

Fig. 3 Dose-response curves displaying the activity of betamethasone (A), beclomethasone (B), budesonide (C), dexamethasone (D), triamcinolone (E), hydrocortisone (F) and doxorubicin (G) in the myeloma cell lines RPMI 8226 and 8226/Dox40 with and without addition of the glucocorticoid-receptor antagonist RU486. The results are presented as mean values ± Standard Errors obtained from 3 to 9 independent experiments

Table 3 IC_{50}-values in micromolar for the glucocorticoids in the cell lines

Cell line	Beclo-methasone	Beta-methasone	Bude-sonide	Dexa-methasone	Hydro-cortisone	Triam-cinolone	Doxo-rubicin
RPMI 8226	> 100	> 100	21.1	> 100	21.1	18.3	0.110
RPMI 8226 + RU486	94.9	81.0	21.3	96.0	33.4	33.0	0.050
8226/Dox40	11.5**	3.9**	1.6*	3.3*	8.50	6*	9.8***
8226/Dox40 + RU486	90.7	84.4	29.9	> 100	34.8	39.7	0.40
8226/LR5	75.7	31.4	0.41**	NA	30.3	8.15	0.071
CCRF-CEM	42.6	24.7	5.04	NA	58.7	27.3	0.064
CEM/VM1	14.3*	2.50**	0.032**	NA	29.2	10.8*	0.748
H69	> 100	85.1	32.9	NA	45.9	32.0	0.092
H69AR	> 100	> 100	7.19	NA	49.0	37.7	1.806***
RF for RPMI 8226 vs 8226/Dox40	8.7	25.6	13.2	30.3	2.48	3.05	0.011
RF for RPMI 8226 vs 8226/LR5	1.32	3.18	52	NA	0.697	2.24	1.54
RF for CCRF-CEM vs CEM/VM1	2.98	9.9	156	NA	2.01	2.53	0.085
RF for H69 vs H69AR	1	0.851	4.58	NA	0.939	0.848	0.051

Student's *t* test was used to compare IC_{50} between the resistant and parental cell lines
NA Not available (the drug was not tested on that cell line)
RF Resistance factor (IC_{50} in the parental divided with IC_{50} in the resistant cell line)
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

to other transcription factors such as AP-1 is of importance in the response [14]. Various co-activators and co-repressors are able to modulate glucocorticoid-receptor-mediated transcription [16]. In the present study only a few of these genes differed in expression between the cell lines, most notably the 11-fold increase in RIP140. However, since RIP140 is known to inhibit, rather than stimulate, glucocorticoid-mediated responses [30], this change in expression cannot explain the glucocorticoid hypersensitivity of the 8226/Dox40 cell line.

To further examine the effect of the glucocorticoid receptor and cofactors on the sensitivity, the glucocorticoid- and progesterone-receptor antagonist RU486 was used to inhibit the effect of the glucocorticoids in the myeloma cell lines, which resulted in a significantly reduced glucocorticoid sensitivity in both RPMI 8226 and 8226/Dox40. This indicates that a functional glucocorticoid receptor is necessary for the cytotoxic activity of the glucocorticoids in the myeloma cell lines. The exact mechanism of glucocorticoid and progesterone antagonism by RU486 has not been resolved in detail [23],

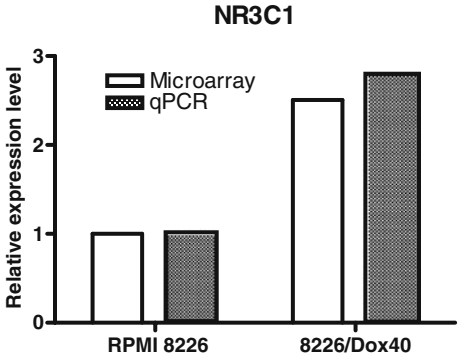

Fig. 4 Relative expression level of the glucocorticoid receptor (NR3C1) in RPMI 8226 and 8226/Dox40. Data are from microarrays and qRT-PCR

Table 4 Fold change in expression of mRNA from 8226/Dox40 compared to mRNA from RPMI 8226

Name	Symbol	Reference sequence	8226/Dox40 vs RPMI 8226
Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	NR3C1	NM_000176	**2.45**
Nuclear receptor co-activator 2	NCOA2	NM_006540	1.41
Nuclear receptor co-activator 3	NCOA3	NM_006534	1.02
Nuclear receptor co-activator 4	NCOA4	NM_005437	1.32
Nuclear receptor co-activator 5	NCOA5	NM_020967	1.15
Nuclear receptor co-activator 6 interacting protein	NCOA6IP	NM_024831	**2.44**
Nuclear receptor co-repressor 1	NCOR1	NM_006311	1.89
Nuclear receptor interacting protein 1	NRIP1	NM_003489	**10.9**
Nuclear receptor interacting protein 2	NRIP2	NM_031474	1.67
Nuclear receptor binding factor 2	NRBF2	NM_030759	1.53
Nuclear receptor binding protein	NRBP	NM_013392	**0.38**
Nuclear receptor binding SET domain protein 1	NSD1	NM_022455	1.53
Proline-rich nuclear receptor co-activator 1	PNRC1	NM_006813	1.13
Proline-rich nuclear receptor co-activator 2	PNRC2	NM_017761	0.73
Transducin (beta)-like 1X-linked receptor 1	TBL1XR1	NM_024665	1.01
E1A binding protein p300	EP300	NM_001429	1.23

however, inhibition of co-activator binding by RU486 has been suggested [31].

Taken together, the present findings suggest that glucocorticoid-receptor expression seems to be, at least partly, a likely explanation for the observed glucocorticoid sensitivity in 8226/Dox40.

The area of phenotype-based screening was recently reviewed [11] and the author concludes that phenotype-based screens have emerged as a key strategy for the identification of novel efficacious drug candidates [11]. In this study, we screened a library of well-characterized compounds in pairs of sensitive and drug-resistant cell lines. The library was chemically diverse and consisted of marketed drugs and failed development candidates and it was possible to find compounds that selectively affected different cellular phenotypes. By comparing several cell types, only the compounds with specific cytotoxicity were chosen for further evaluation. Pharmacodynamic and safety information in man already exists for many drugs, making the way to clinical tests shorter.

In conclusion, screening of annotated compounds on drug-resistant cancer cells can identify compounds with selective activity in resistant cells which can provide a basis for the development of novel therapies for drug-resistant malignancies.

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