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Understanding drugs in breast cancer through drug sensitivity screening

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Abstract

With substantial numbers of breast tumors showing or acquiring treatment resistance, it is of utmost importance to develop new agents for the treatment of the disease, to know their effectiveness against breast cancer and to understand their relationships with other drugs to best assign the right drug to the right patient. To achieve this goal drug screenings on breast cancer cell lines are a promising approach. In this study a large-scale drug screening of 37 compounds was performed on a panel of 42 breast cancer cell lines representing the main breast cancer subtypes. Clustering, correlation and pathway analyses were used for data analysis. We found that compounds with a related mechanism of action had correlated IC_{50} values and thus grouped together when the cell lines were hierarchically clustered based on IC_{50} values. In total we found six clusters of drugs of which five consisted of drugs with related mode of action and one cluster with two drugs not previously connected. In total, 25 correlated and four anti-correlated drug sensitivities were revealed of which only one drug, Sirolimus, showed significantly lower IC_{50} values in the luminal/ERBB2 breast cancer subtype. We found expected interactions but also discovered new relationships between drugs which might have implications for cancer treatment regimens.

Keywords: Drugs, Screening, Cell line, Subtype, Pathway, Breast cancer

Background

Life expectancy and survival of breast cancer patients have increased significantly over the last decades, due to—amongst other factors—an increasing number of effective drug therapies (Berry et al. 2005; Lichtenberg 2009, 2011). Drug resistance remains a major issue (Gonzalez-Angulo et al. 2007) and since the discovery that expression of the protein markers ER, PR and her-2/neu determines response to a given targeted therapy (Bast et al. 2001), the assessment of their expression in breast cancer has become an important first step in selecting a patient's treatment (Bast et al. 2001). Subsequently, microarray studies have shown insight into molecular processes active in the tumor and linked those to diverse clinical outcomes (Sorlie et al. 2001; Van't Veer et al. 2002; Wang et al. 2005) including therapy failure (Jansen et al. 2005). In the last couple of years large scale next generation sequencing efforts have made a big contribution to our understanding of breast cancer by delivering precise information on cancer driver mutations (Kangaspeska et al. 2012; Desmedt et al. 2012; Previati et al. 2013; Radovich et al. 2013; The Cancer Genome Atlas Network 2012). All these sources of information combined have helped to elucidate how breast cancer evolves, progresses and metastasizes and some of them have led to the development of diagnostic tests to characterize breast cancer better (Kittaneh et al. 2013). Nevertheless, there is still significant room for improvement in regard to available drug therapies, as many patients do not respond to current treatments or become resistant during the course of treatment (Gonzalez-Angulo et al. 2007). New agents are therefore needed to target breast cancer, and screenings of multiple compounds for their activity against the various breast cancer subtypes are a good starting point. As a first step to test new compounds breast cancer cell lines are a good model, because

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they are easy to maintain, represent different subtypes of breast cancer, and the response to drug treatment can be easily assessed. For these reasons, we studied the activity of a wide variety of cytotoxic and targeted drugs in a large panel of breast cancer cell lines. The drugs were chosen based on current clinical utility e.g. for discrete cancer subtypes, potential clinical utility such as promising compounds in pre-clinical testing, aiming at molecular targets, and—for comparison—current state of the art drugs for the therapy of breast cancer. We investigated which drugs showed similar activity in the panel of breast cancer cell lines and could therefore potentially substitute or complement each other in the clinic, and, in addition, we aimed to identify shared biology in cell lines that are affected by highly correlated drugs.

Results

Relationships between drugs: clustering and correlation analysis

To investigate the relationships between different drugs the IC_{50} values of all 7 cytotoxic drugs and 30 targeted agents, measured in the 42 breast cancer cell lines, were correlated (Fig. 1). Capecitabine, cMet 605 and Cyclophosphamide exhibited no differential IC_{50} values and were consequently omitted from the clustering and further analyses. To express the relationships among drugs and cell lines hierarchical clustering was performed (Fig. 2). Clustering and correlation performed fairly similarly and are therefore discussed together.

Strong correlation and expected co-clustering was observed between Gefitinib and Erlotinib (cluster 1; $r = 0.88$), between Quisinostat, Panobinostat, Vorinostat and Belinostat (cluster 2; $r = 0.85$–0.96), between Docetaxel and Paclitaxel (cluster 3; $r = 0.73$), between JNJ-707 and JNJ-493 (cluster 4; $r = 0.62$) and between MI-219 and Nutlin-3 (cluster 6; $r = 0.98$); all correlations are listed additionally in Table 1. To illustrate the close relationship between related drugs, the IC_{50} values of MI-219 and Nutlin-3, the two drugs with the highest correlation, were ranked and plotted for all cell lines (Fig. 3). Interestingly, Serdemetan, a drug which acts on cholesterol transport but also targets MDM2 (Jones et al. 2013)—a mechanism shared with Nutlin-3 and MI-219 (Shangary and Wang 2009)—showed no correlation with these two compounds.

Unanticipated but highly significant correlations were observed between particularly Doxorubicin and Azacitidine (cluster 5; $r = 0.70$), between Decitabine and 5-Fluorouracil ($r = 0.58$) and Serdemetan ($r = 0.56$); and between Serdemetan and Tipifarnib ($r = 0.52$). Additional weaker, but expected correlations were found for Vandetanib with Erlotinib and Gefitinib ($r = 0.47$, $r = 0.56$). Decitabine was correlated with Lapatinib ($r = 0.52$) and Veliparib with Serdemetan and Decitabine ($r = 0.51$, $r = 0.48$). Furthermore, we also detected a remote relation between various tyrosine kinase inhibitors like JNJ-493 with the multi-receptor tyrosine kinase inhibitor Sunitinib (Keyvanjah et al. 2012) ($r = 0.48$), JNJ-707 with FGFR- and VEGFR-inhibitor Brivanib (Huynh et al. 2008) ($r = 0.46$) and between Docetaxel and ARQ197 ($r = 0.47$). The DNA targeting drug Cisplatin (Becker et al. 2014) showed surprisingly a correlation with Sunitinib ($r = 0.47$); Bortezomib was correlated with Vandetanib ($r = 0.47$) and the type II topoisomerase inhibitor Mitoxantrone (Hajihassan and Rabbani-Chadegani 2009) was correlated with JNJ-707 ($r = 0.45$). In total, 25 pairs of positively correlated drugs were found.

Apart from positive correlations—and even more interesting—we also discovered significant negative correlations between certain drugs (Table 1). Particularly, Doxorubicin and the correlated drug Azacitidine had negative correlations with Cisplatin ($r = -0.73$, $r = -0.55$), the ERR1 targeting JNJ-208 with Bortezomib ($r = -0.66$), and the MDM2-targeting Nutlin-3 (Shangary and Wang 2009) with the FGFR inhibitor JNJ-707 ($r = -0.45$).

Shared pathways between correlated drugs

To further understand the biology behind correlated drugs we used mRNA expression data of the untreated cell lines and the pathway information of the databases Biocarta and KEGG (Ogata et al. 1999) to characterize drug resistance in R (R_Core_Team 2013). We identified significant pathways for each of the evaluable drugs, but focused on the pathways which were shared among correlated drugs, i.e. for the 23 positively and 3 negatively correlated remaining drug–drug pairs. Furthermore, we performed a pathway analysis where cell lines were grouped per subtype to identify subtype-related pathways. Subtype-specific pathways were excluded from further study in the pathway-drug resistance analysis. At a significance level of $p < 0.01$, only one of all 26 correlation pairs had pathways in common. This pair, Nutlin-3 and MI-219, had, after correction for subtype-specific pathways, only the DNA replication pathway in common. The Nutlin-3- and MI-219-associated genes of this pathway are displayed in Fig. 4.

Breast cancer subtype specific drugs

Earlier, several subtype-specific differences in drug sensitivity were observed (Heiser et al. 2012) and since breast cancer subtypes are biologically very different (Parker et al. 2009), we also explored whether drug response in our study was ER- or subtype-related. Only one drug,

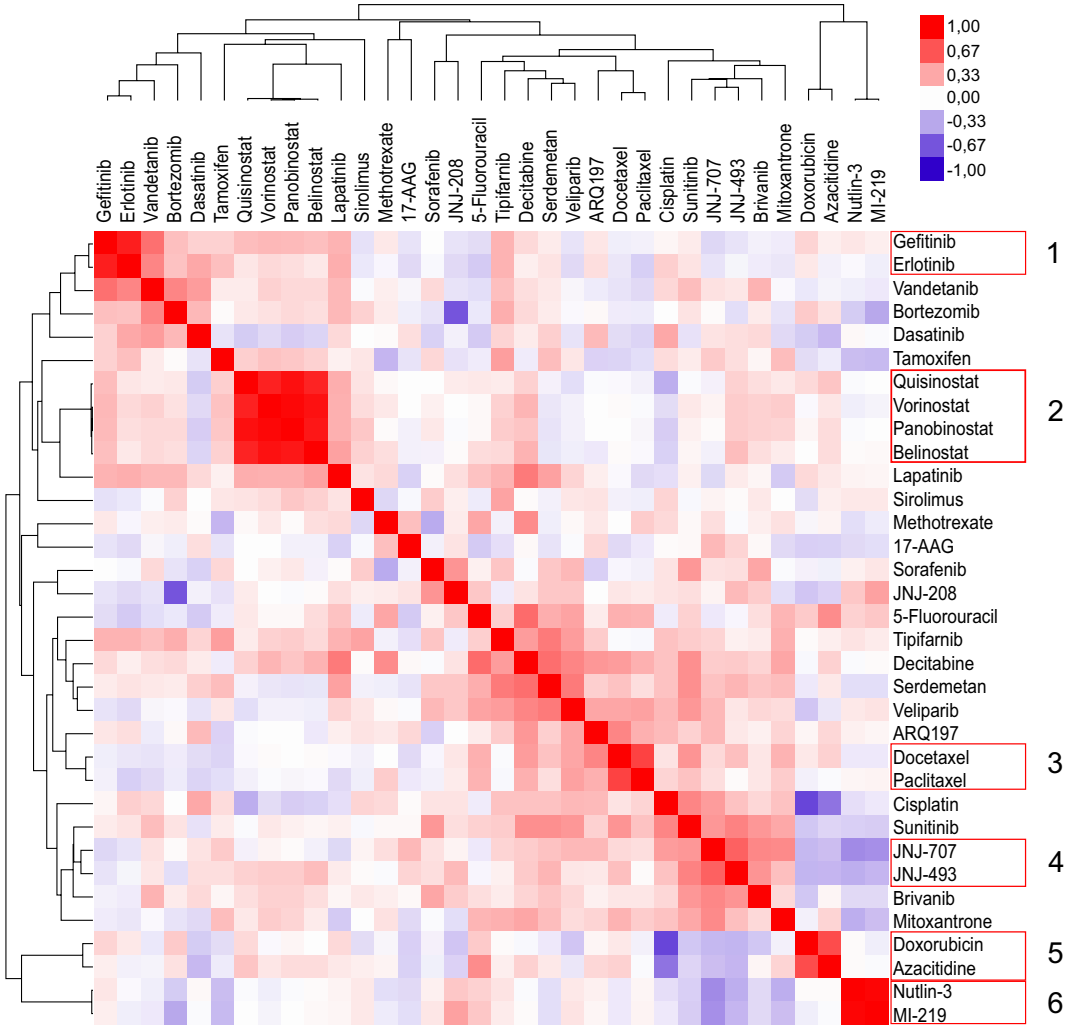

Fig. 1 Pearson correlation plot of absolute drug IC_{50} values. The *red color* indicates a positive correlation between the IC_{50} values of two drugs, and *blue* a negative correlation. The *color intensity* illustrates the correlation coefficient as shown in the legend at *top right*. Drugs are clustered on the basis of similarity; distances in the tree indicate the degree of difference between drugs

Sirolimus, exhibited a significantly different subtype-specific effectiveness. Normal-like and basal cell lines were more resistant to this drug compared to luminal and ERBB2-overexpressing cell lines with a change in sensitivity of two orders of magnitude ($p = 0.005$). The expression of ER by the latter two subtypes was not the sole explanation though, as none of the screened drugs was associated with ER status (p value >0.01).

Discussion

Drug response to one drug indicates the response to another

To understand drug resistance in breast cancer, we compared drug sensitivity of a large set of drugs within a large panel of breast cancer cell lines. It became evident that some drugs target breast cancer cell lines similarly and thus may have unanticipated overlapping mechanisms while others display opposing effects indicating that vulnerability to a given drug is protective for another unrelated treatment.

The results of the overall clustering (Fig. 2) show that every breast cancer cell line had a unique drug response profile, which might be true for patients as well. This—first—observation underlines the personal factor in drug sensitivity, which we need to understand upfront to provide optimal patient care.

The second, expected, conclusion is that drugs with identical targets such as MDM2-antagonists (MI-219 and Nutlin-3) (Shangary and Wang 2009), EGFR-inhibitors (Gefitinib and Erlotinib) (Cohen 2003), FGFR-inhibitors

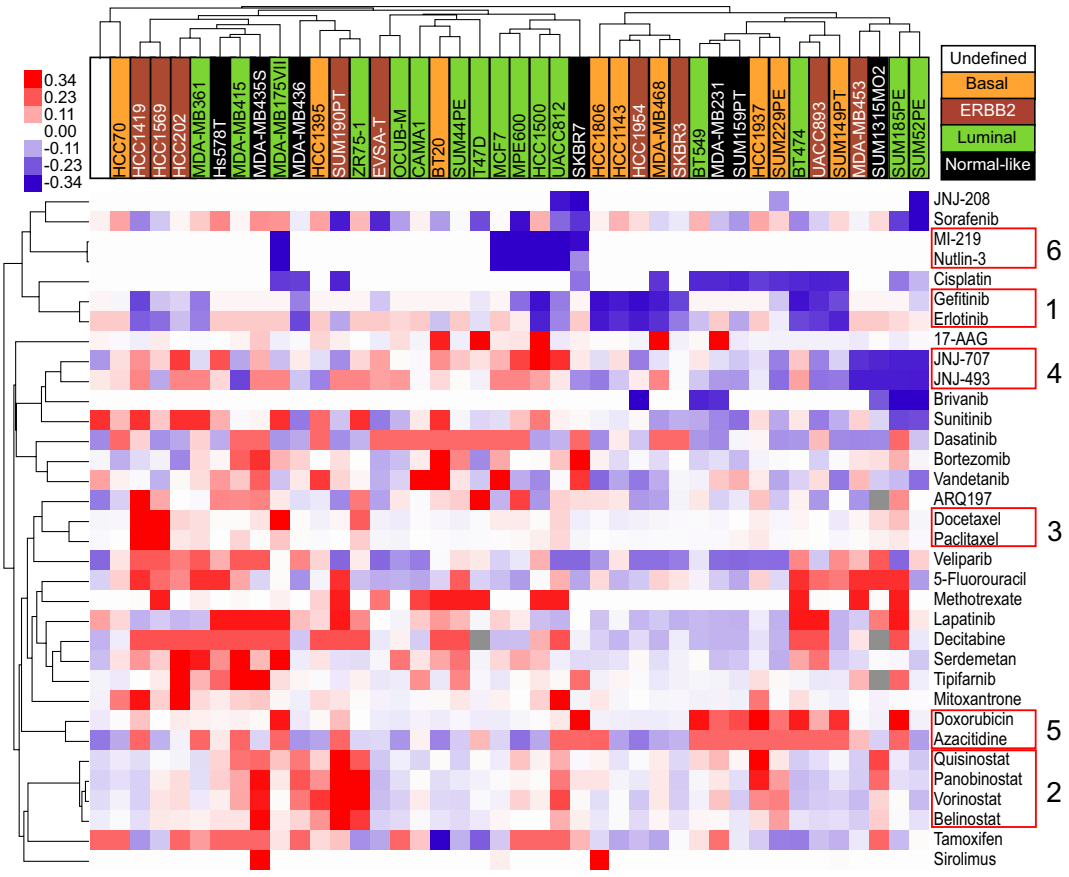

Fig. 2 Similar drugs cluster together. Depicted is a hierarchical unsupervised clustering of the analyzable drugs and cell lines. *Blue color* indicates low IC_{50} values (i.e. cells are drug-sensitive), and *red color* high IC_{50} values (i.e. cells are drug-resistant). *Color intensity* illustrates the degree of drug sensitivity or resistance; outliers exceeding the legend boundaries are set to the maxima colors of the legend to ensure visibility of small differences instead of few outliers. Breast-cancer subtypes are color-coded on the basis of the intrinsic subtypes of breast cancer cell lines as previously described (Riaz et al. 2013). The respective legend can be found on the *top right*. Tree distance is representative for similarity of drugs or cell lines. Drugs with similar response profiles among the cell lines are highlighted by *red boxes*

(JNJ-707 and JNJ-493), HDAC inhibitors (Quisinostat, Panobinostat, Vorinostat, Belinostat) (Lemoine and Younes 2010) and taxanes (Docetaxel and Paclitaxel) (Hagiwara and Sunada 2004), showed correlated sensitivities and clustered together explaining five of the six observed clusters.

More interesting was the third observation that unrelated drugs showed co-clustering, which is best exemplified by the sixth cluster (Figs. 1, 2), made up of the positively correlated intercalating agent Doxorubicin (Frederick et al. 1990) and the DNA-methyltransferase-targeting Azacitidine (Creusot et al. 1982). Interestingly and remarkably, Decitabine, a derivative of Azacitidine (Lyko and Brown 2005), which also targets a DNA-methyltransferase (Creusot et al. 1982), did not cluster with these two drugs. The reason for this might be that both Azacitidine and Doxorubicin have, next to their well-known properties, also the less known capability to interfere with RNA synthesis (Momparler et al. 1976; Christman 2002), while Decitabine can only act on DNA (Christman 2002). Next to this most notable finding we also observed a less strong correlation of Decitabine sensitivity with sensitivity to various unrelated drugs, i.e. the thymidylate synthetase inhibitor 5-Fluorouracil (Longley et al. 2003), the cholesterol transport inhibitor and MDM2-antagonist Serdemetan (Lehman et al. 2013; Jones et al. 2013), the EGF-receptor- and HER2-inhibitor Lapatinib (Huang and Rizzo 2012) and the PARP-inhibitor Veliparib (Glendenning and Tutt 2011). Some of these drugs additionally correlated with each other. Although several of these compounds target DNA synthesis and/or repair, there is no real common denominator between them. While these drugs could be targeted by the same drug efflux pumps, we could not find any among the drug-associated genes

Table 1 Correlated drugs

Drug 1	Drug 2	p-value	Pearson correlation coefficient
MI-219	Nutlin-3	1.77E−28	0.98
Panobinostat (Faridak®)	Vorinostat (Zolinza®)	2.14E−24	0.96
Panobinostat (Faridak®)	Quisinostat	1.66E−19	0.93
Belinostat	Vorinostat (Zolinza®)	2.05E−18	0.92
Belinostat	Panobinostat (Faridak®)	1.70E−16	0.91
Erlotinib (Tarceva®)	Gefitinib (Iressa®)	3.49E−14	0.88
Quisinostat	Vorinostat (Zolinza®)	1.05E−13	0.87
Belinostat	Quisinostat	8.08E−13	0.85
Paclitaxel (Taxol®, OnxalTM)	Docetaxel (Taxotere®)	4.61E−08	0.73
Azacitidine (Vidaza®)	Doxorubicin (Adriamycin®)	3.77E−07	0.7
JNJ-493	JNJ-707	1.39E−05	0.62
Decitabine (Dacogen®)	5-Fluorouracil	7.77E−05	0.58
Decitabine (Dacogen®)	Serdemetan	1.17E−04	0.56
Vandetanib (Zactima®)	Gefitinib (Iressa®)	1.52E−04	0.56
Serdemetan	Tipifarnib (Zarnestra®)	5.15E−04	0.52
Decitabine (Dacogen®)	Lapatinib	5.29E−04	0.52
Veliparib	Serdemetan	5.47E−04	0.51
JNJ-493	Sunitinib (Sutent®)	1.37E−03	0.48
Veliparib	Decitabine (Dacogen®)	1.63E−03	0.48
Vandetanib (Zactima®)	Erlotinib (Tarceva®)	1.78E−03	0.47
Bortezomib (Velcade®)	Vandetanib (Zactima®)	1.94E−03	0.47
ARQ197	Docetaxel (Taxotere®)	1.95E−03	0.47
Cisplatin	Sunitinib (Sutent®)	2.00E−03	0.47
JNJ-707	Brivanib	2.16E−03	0.46
Mitoxantrone (Novantrone®)	JNJ-707	2.98E−03	0.45
JNJ-707	Nutlin-3	2.87E−03	−0.45
Cisplatin	Azacitidine (Vidaza®)	2.16E−04	−0.55
JNJ-208	Bortezomib (Velcade®)	1.96E−06	−0.66
Cisplatin	Doxorubicin (Adriamycin®)	5.22E−08	−0.73

Correlation pairs were determined using IC_{50} values. Statistical thresholds for significance were defined as a p-value <0.01 and a Pearson correlation coefficient above 0.45 or below −0.45

(pre-treatment gene expression) and suspect another, unknown mechanism. The same holds true for the associations of Serdemetan with Veliparib and the farnesyltransferase inhibitor Tipifarnib (Armand et al. 2007).

Additionally, we also observed a rather surprising correlation between Docetaxel, which disorganizes microtubules (Hagiwara and Sunada 2004), and the c-met kinase inhibiting agent ARQ197 (Scagliotti et al. 2013). However, supporting our findings, ARQ197 has also been linked to inhibition of microtubuli polymerization recently (Katayama et al. 2013).

Finally, sensitivity to Bortezomib, a proteasome inhibitor (Teicher et al. 1999), was also predictive of sensitivity to the multi-kinase inhibitor Vandetanib (Sathornsumetee and Rich 2006)—the combination of these two drugs is currently in clinical trial testing (Gramza et al. 2013). Thus, a protein or complex whose stability is proteasome-dependent, may affect sensitivity to this multi-kinase inhibitor. Furthermore, Cisplatin and JNJ-493 were somewhat correlated to Sunitinib sensitivity and JNJ-707 weakly correlated with response to Mitoxantrone, findings which remain to be understood.

The fourth finding was the absent or poor correlation of drugs acting on the same target, such as Serdemetan which was not correlated with the other two well-known and highly correlated MDM2-inhibitors Nutlin-3 and MI-219 (Shangary and Wang 2009). Furthermore, Serdemetan lacked a correlation with TP53 mutation status (data not shown) highlighting that this putative MDM2-inhibitor acts differently from the other MDM2-inhibitors. This unexpected observation can be explained by the additional characteristics of Serdemetan, inhibition of the cholesterol

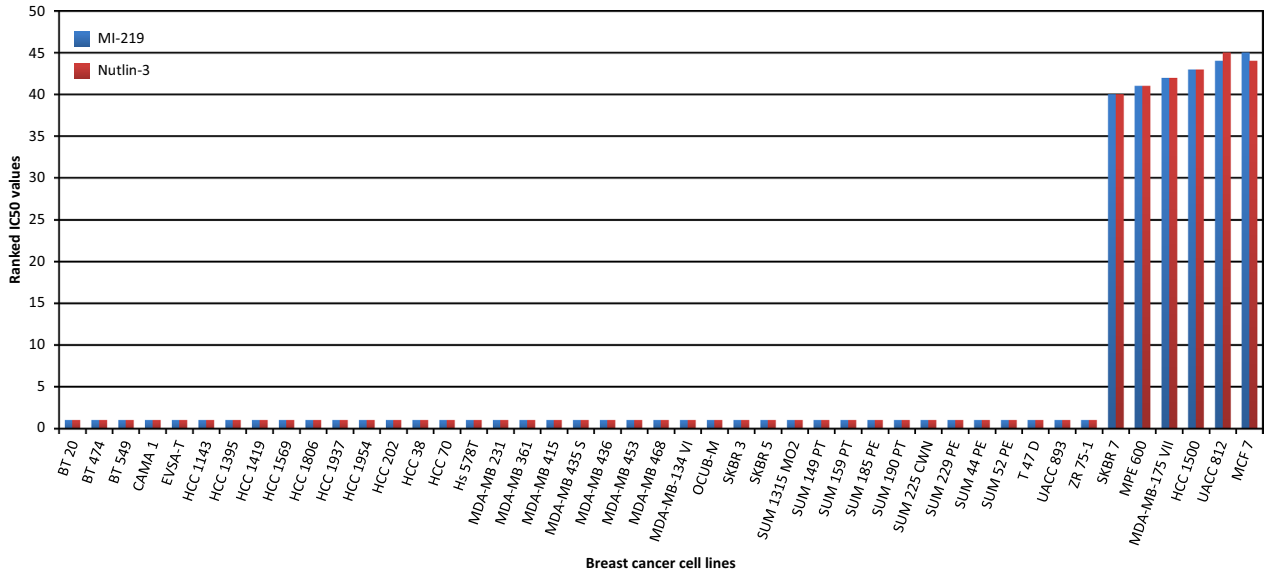

Fig. 3 Nutlin-3 and MI-219 have similar drug sensitivity profiles among the cell lines (relative IC_{50} values). The relative IC_{50} is inverted, with high numbers indicating sensitivity in this case and not resistance. Few cell lines are sensitive to these drugs, while the majority is resistant

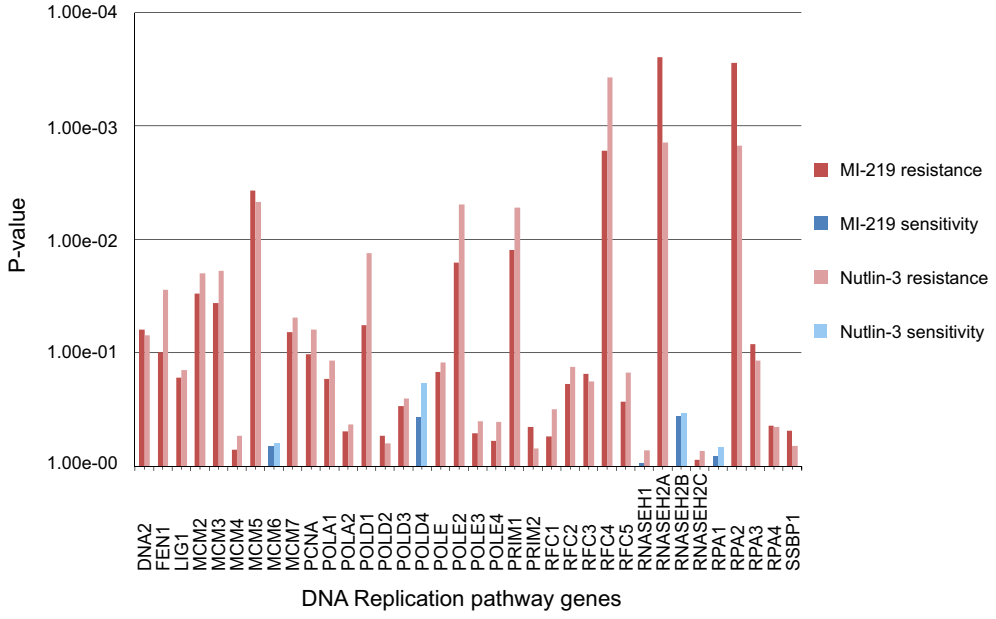

Fig. 4 Differentially expressed genes of the DNA replication pathway for Nutlin-3 and MI-219. *Bar graphs* display the differentially expressed genes of this pathway between resistant and sensitive cell lines for Nutlin-3 and MI-219. *Red shades* indicate an association with resistance, *blue shades* indicate an association with sensitivity

transport and the increased degradation of ABCA1 (Jones et al. 2013). Clearly these additional properties dominate over the MDM2-inhibiting role. Moreover, another study confirms that sensitivity to Serdemetan is independent of TP53-mutation status (Jones et al. 2013).

Furthermore, we noted that Lapatinib and Vandetanib, two EGFR-antagonists (Nelson and Dolder 2006; Sathornsumetee and Rich 2006) neither clustered immediately next to Gefitinib or Erlotinib nor next to each other and the correlation coefficient was also lower than expected

for Vandetanib, while Lapatinib did not correlate with the other EGFR-antagonists at all. In both cases this is less surprising as both Vandetanib and Lapatinib act on additional targets, like HER2 for Lapatinib (Nelson and Dolder 2006), and the two proteins VEGFR2 and RET Kinase for Vandetanib (Sathornsumetee and Rich 2006). We tried to support this hypothesis by correlating the drug response with mRNA expression data of EGFR, HER2, FGFR, VEGFR2, and RET Kinase for all those drugs. However, none of the correlations was significant, which might be different if pre-treatment protein expression data is used, as the proteins are the direct drug targets.

Similarly, Brivanib showed only a weak correlation with one of the FGFR-inhibitors, JNJ-707, in our panel, which might be due to its additional target VEGFR (Huynh et al. 2008).

Drug response to one drug indicates resistance to another

The fifth interesting observation was that for some drugs sensitivity predicted insensitivity to another drug. For instance—and most prominent—Cisplatin resistance correlated with Doxorubicin sensitivity. Cisplatin's mode of action involves DNA and RNA interstrand linkage (Stordal and Davey 2007), while Doxorubicin blocks DNA unwinding (Fornari et al. 1994), this difference does however not explain the clearly opposing character in drug response. The clinical implication might, nevertheless, be, that a patient showing insensitivity to Doxorubicin upon treatment start, might be more likely to respond to a course of Cisplatin therapy (Perilongo et al. 2009). Another implication of this finding is that a mechanism responsible for resistance to Doxorubicin reveals a target that provides synthetic lethality to Cisplatin or vice versa.

Previously, Cisplatin resistance was found to correlate with Taxane sensitivity (Stordal et al. 2007), a finding, we could not confirm in the present study.

The FGFR inhibitor JNJ-707 had an inverse correlation with Nutlin-3 response. Therefore, we also investigated whether TP53 wild-type cell lines (Riaz et al. 2013), which are sensitive to Nutlin-3, have a different expression of FGFR genes in contrast to mutant cell lines, but found no significant difference (data not shown). While a true biological effect cannot be excluded, it has to be mentioned that only few cell lines were sensitive to Nutlin-3 and our observation might be due to the low numbers. Finally, the proteasome inhibitor Bortezomib is negatively correlated to JNJ-208. Thus, if this observation implies causality, a proteasome-dependent mechanism affects vulnerability to this drug.

Biology of correlated drugs

Next, we wanted to uncover shared biology of drug sensitivity in correlated drugs by performing a pathway analysis. The first thing we discovered was that there were hardly any shared pathways after excluding pathways with a strong subtype-association. This was rather surprising as we did not find many subtype-associated drugs, but can be explained by the analysis type since for the pathway analysis we used only the cell lines around the minimum and maximum drug response, while for the subtype analysis all cell lines were included. Hence the smaller number of cell lines might have introduced a bias, but generally, it seems that the biology driving the subtypes in breast cancer largely obscures the possible drug-related pathways. The two correlated drugs which had the DNA replication pathway in common were Nutlin-3 and MI-219. Nutlin-3 has been previously shown to downregulate proteins involved in DNA replication (Kumamoto et al. 2008), a process likely influenced by MI-219, as well. The cell lines which were sensitive to these drugs had intrinsically low expression of most pathway-associated genes pre-treatment and the drug-related shutdown of the remaining expression might be contributing to lethality.

From the results of the subtype-specific pathways it became obvious that normal-like cell lines are very different from luminal ones. However, when we did a global test to evaluate whether a certain breast cancer subtype showed an overall increased sensitivity to the tested compounds we found no differences. Therefore, we could not confirm a general drug resistance of normal-like cells which would be expected due to their mesenchymal and stem cell like properties (Al-Hajj et al. 2003; Ponti et al. 2006; Sieuwerts et al. 2009).

Subtype-specific drugs

Of all tested drugs in this screening only one drug, Sirolimus, was more active in the luminal and ERBB2-high subtypes, as was noted previously in a comparable study (Heiser et al. 2012). However, in contrast to this earlier study (Heiser et al. 2012), who discovered 23 subtype-related drugs, we did not find subtype-dependent sensitivity for the other eight drugs screened in both studies. This discrepancy is likely due to several differences in study design, e.g. drug incubation times, type of read-out assay, the use of collagen-coated plates in our study to mimic cellular context better and differences in the assayed cell lines to name a few.

Conclusion

Through our cell line screening with new and well-known drugs, we found a number of interesting interactions between drugs of which several were not noticed earlier. Those findings have great potential for an application in the clinic as they might present opportunities

when tumors show already resistance upon start of the treatment.

Next to expected similar sensitivity profiles for related drugs such as Gefitinib and Erlotinib, we also found opposing sensitivity profiles such as Cisplatin with Doxorubicin and confirmed one subtype-related drug, Sirolimus, which has been identified earlier. Further validation on the discovered positive and negative correlations and the subtype-specific drug are needed e.g. in the form of an animal study. In that aspect it would be very interesting to investigate whether animals with e.g. a Cisplatin-resistant tumor benefit from Doxorubicin treatment.

To conclude, our study provides new leads in the search for effective treatments especially in the context of inherent drug resistance.

Methods

Cell lines and drug screening

Forty-five breast cancer cell lines with confirmed identity (Riaz et al. 2013) and known origin (Hollestelle et al. 2010a) were cultured and screened in RPMI 1640 medium (Life Technologies, Paisley, UK) containing 10 % FBS (Lonza, Walkersville, USA). Ninety-six well collagen I-coated plates (BD Biosciences, San Jose, USA) were used for drug screening. Each drug—cell line combination was assayed in triplicate. Cells were seeded at the density required to reach the end of the exponential growth phase at 120 h of culture. Drug incubation was started 24 h post-seeding and lasted 96 h. For each drug 12 different dilutions were tested starting from 1.00^{-5} to 3.00^{-11} M (final concentration), except for Bortezomib (2.00^{-5} to 6.00^{-11} M), Sirolimus and 17-AAG (both: 2.00^{-6} to 6.00^{-12} M). Drug diluent was used as negative control treatment. DMSO was used as drug solvent and diluent for all drugs except for Methotrexate (drug solvent: 1 M NaOH, drug diluent: 0.9 % NaCl solution) and Cyclophosphamide (drug solvent: PCR-grade water, drug diluent: 0.9 % NaCl solution).

Three cell lines, SUM225CWN, MDA-MB-134VI, and SKBR5, failed in our drug screening due to slow growth or half-suspension growth, which is incompatible with the SRB assay, resulting in 42 cell lines for analysis.

Assessment of drug sensitivity and IC_{50} calculation

Cell line growth was determined by measuring the total protein amount per well using the Sulforhodamine B Assay (SRB) (Voigt 2005): After the medium was discarded the cells were incubated with 10 % TCA for 60 min at 4 °C for fixation. Then the cells were thoroughly washed 5× with distilled water, air-dried and incubated with 0.4 % SRB solution for 2 h for protein-staining. Additional washing steps followed using 1 % acetic acid (4×) and cells were again air-dried. TRIS (10 mM) was added to the cells to dissolve the SRB overnight. Absorbances were measured at 570 nm in an Ascent MultiSkan (Thermo Electron Corporation, Waltham, USA). If required, samples were further diluted with TRIS to ensure optimal measurements. IC_{50} values were calculated using the respective absorbance values and are listed, besides all IC_{50} profiles, in the supplemental Excel file (Additional file 1).

Clustering of cell lines and drug–drug correlation analyses

Cell lines were clustered in a hierarchical fashion based on their IC_{50} values. For drug correlation analysis, IC_{50} values per compound of each cell line were correlated with each other in Excel 2007 (Microsoft, Redmond, USA) using Pearson correlation. Cell lines with missing data for several drugs were discarded from correlation and cluster analysis, as were drugs that did not show differential IC_{50} values. The programs Cluster 3.0 (Eisen Lab, Stanford University, Stanford, USA) and Java Treeview version 1.1.6r2 (Saldanha 2004) were used to generate heatmaps of the correlation coefficients and the cluster analysis (Figs. 1, 2). Cluster 3.0 settings were as follows: normalize, median—center and average linkage using uncentered correlation as similarity metric. Figure 3 was generated in Excel. To generate the figures Inkscape 0.91 (Free Software Foundation Inc., Boston, USA) was used, next to the aforementioned programs.

Association of signaling pathways with drug response

For pathway analysis we analyzed gene expression levels of cell lines with a high IC_{50}- versus cell lines with a low IC_{50}-value per drug. Cell lines were grouped in the high or low group by individual evaluation per drug instead of pre-selecting a fixed IC_{50} value for all drugs. This method was chosen to test the drug response extremes rather than testing values with little difference, which are present in gradual IC_{50} distributions. For this distribution type we used the cell lines at the distribution extremes and removed the intermediate values to reduce noise. A few drugs showed an IC_{50} profile that precluded a sensible grouping; e.g. Paclitaxel had only two cell lines with a high IC_{50} value while the others had a very low IC_{50}; too few cell lines in a group renders the pathway analysis useless. For this reason, we excluded JNJ-208, Sirolimus, Docetaxel and Paclitaxel from the pathway analysis. For all other drugs, we were able to use meaningful group sizes of at least five samples each (Additional file 1).

For all cell lines, previously published mRNA expression data of our laboratory (Riaz et al. 2013) was used for pathway analysis (NCBI's Gene Expression Omnibus database, entry GSE41313). Pathway analysis was performed using the Global Test package (Goeman et al. 2004) in R (R_Core_Team 2013) with information of the

databases BioCarta LLC (San Diego, USA) and KEGG (Ogata et al. 1999). This R package was also used to generate Fig. 4, next to Excel 2007 and Inkscape 0.91. Furthermore, we also tested the identified pathways for a stronger association with breast cancer subtypes and disregarded those subtype-associated pathways (Additional file 2). The pathways significantly associated with a drug, including the subtype-associated ones, are listed in Additional file 2.

Association of drug response with breast cancer subtype and ER status

All drugs were tested for association with breast cancer cell line subtypes. First, cell lines were grouped into luminal, basal, ERBB2-overexpressing and normal-like on the basis of intrinsic subtypes as previously reported (Hollestelle et al. 2010b). Statistical testing was performed either in BRB Array Tools version 4.2.1 Class Comparison using a T test or in Analyse-it version 2.26 (Leeds, UK) for Chi Square tests. To test whether ER protein expression was significantly associated with drug response, a Mann–Whitney test was used for linear IC_{50} profiles and a Chi Square or Fisher's Exact test for two-group IC_{50} profiles. Previously published ER protein expression data (Riaz et al. 2013) was used as a categorical variable for ER status.

Additional files

Additional file 1. Detailed results of the drug screening and additional information regarding the data analysis. This table contains all calculated IC_{50} values for each drug and cell line. Furthermore the plotted IC_{50} values per drug are displayed and the categorization of the cell lines into resistant and sensitive per drug for the pathway analysis are listed.

Additional file 2. Pathways associated with drugs. This file lists all Biocarta and KEGG pathways that are significantly associated with at least one drug. Pathways that are more significantly associated with a breast cancer subtype are in italic. The p-values given are for the drug-pathway association.

Abbreviations

ABCA1: ATP-binding cassette, sub-family A (ABC1), member 1; c-Met: MET proto-oncogene; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; EGFR: epidermal growth factor receptor; ER: estrogen receptor; ERBB2: Erb-b2 receptor tyrosine kinase 2; ERR1: estrogen-related receptor alpha; FBS: fetal bovine serum; FGFR: fibroblast growth factor receptor; HDAC: histone deacetylase; HER2: human epidermal growth factor receptor 2, Erb-b2 receptor tyrosine kinase 2; Her2-/neu: Erb-b2 receptor tyrosine kinase 2; IC_{50}: inhibitory concentration 50, half maximal inhibitory concentration; KEGG: Kyoto Encyclopedia of Genes and Genomes; MDM2: MDM2 proto-oncogene, E3 ubiquitin protein ligase; NaCl: sodium chloride; NaOH: sodium hydroxide; NCBI: National Center for Biotechnology Information; PARP: poly ADP ribose polymerase; PR: progesterone receptor; RET: ret proto-oncogene; RNA: ribonucleic acid; RPMI: Roswell Park Memorial Institute; SRB: sulforhodamine B; TCA: trichloroacetic acid; TP53: tumor protein p53; TRIS: tris(hydroxymethyl)aminomethane; VEGFR: vascular endothelial growth factor receptor, kinase insert domain receptor; VEGFR2: vascular endothelial growth factor receptor 2, kinase insert domain receptor.

Authors' contributions

KU, JWMM and JAF were involved in writing this article. WJCPvdS and BO performed the cell culture and drug screening. MS, AAJH, KU and JWMM interpreted the data and were involved with the statistical analysis. HWHG, JWMM and JAF were involved with the design of the study, AJ contributed to the design of the study. All authors read and approved the final manuscript.

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Competing interests

Janssen Pharmaceutica (Beerse, Belgium) initially funded this drug screening as contract research with the possibility to use the results later for academic research. HWHG is an employee of Janssen Pharmaceutica and was involved in this study as collaboration partner from Janssen Pharmaceutica. JWMM and JAF received funds from Janssen Pharmaceutica. AJ is involved as local PI in clinical trials on Veliparib and other compounds of AbbVie (North Chicago, USA). The authors KU, WJCPvdS, AAJH, BO, MS declare that they do not have competing interests.

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