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# Broad-spectrum Cross-resistance to Anticancer Drugs Mediated by Epidermal Growth Factor Receptor

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**Abstract.** *Background: The oncogenic role of epidermal growth factor receptor (EGFR) has been intensively studied. However, its emerging role in drug resistance has not been fully addressed. Materials and Methods: This study systematically investigated the correlation of mRNA and protein expression of EGFR, as well as gene amplification and mutations with the log-transformed half-maximal inhibitory concentration ($log_{10}IC_{50}$) values obtained from the NCI panel of 60 human tumor cell lines against 83 standard anticancer agents and the top 10 natural cytotoxic products previously screened by us. Results: EGFR protein expression, rather than other measurements, was most frequently associated with drug response. $Log_{10}IC_{50}$ and EGFR protein level were significantly positively correlated under all investigated DNA topoisomerase (TOPO) II inhibitors, followed by 81% of alkylating agents and platinum-based compounds, 71% of anti-hormones, 66% of TOPO I inhibitors and 50% of antibiotics. Furthermore, 60% of cytotoxic natural products did not reveal significant correlations. Conclusion: Collectively, we showed a broad-spectrum of cross-resistance towards clinical drugs mediated by EGFR. Natural cytotoxic products may be further developed as novel drugs to overcome EGFR-associated resistance to clinically established anticancer drugs.*

The epidermal growth factor receptor (EGFR) is an oncogenic receptor tyrosine kinase (RTK), which is commonly overexpressed or hyper-activated in solid tumors. Its downstream cascades upon activation are involved in carcinogenesis (1). Consequently, EGFR-driven therapeutic strategies have developed rapidly since the early 1990s (2). The tyrosine kinase activity of EGFR represents a successful therapeutic target. Three generations of tyrosine kinase inhibitors have been brought onto the market, *e.g.* gefitinib, afatinib and osimertinib. Despite the initial clinical benefit, many patients eventually experience disease progression due to mutation or alternative pathway activation (3, 4). Another pharmacological approach for impeding EGFR activation is the use of neutralizing monoclonal antibodies, *e.g.* cetuximab, panitumumab and nimotuzumab (5-7). These antibodies can induce antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity (8, 6). There are also studies demonstrating that these antibodies trigger internalization and thereafter degradation of EGFR, which further down-regulates the total EGFR level (9,10).

In addition to its role as a prominent anticancer target, EGFR and its downstream signaling molecules are also considered to be emerging determinants of drug resistance to first-line chemotherapies and ironizing radiotherapy (11-13). Activation of EGFR-relevant molecules provide compensatory pro-survival pathways for cancerous cells under the effect of anticancer therapies. Notably, EGFR internalization to the nucleus was also believed to induce resistance to radiotherapy, DNA-damaging agents and EGFR-targeted therapy by different mechanisms (14-17). Recently, another finding has led to the argument that nuclear EGFR upon exposure to stress modulates the stability and translation of microRNA (miRNA)-regulated mRNAs, which enables cells to express proteins directly in response to EGFR activation. This may also contribute to resistance in EGFR-overexpressing tumors (18). In this study, we correlated *EGFR* mRNA and EGFR protein expression, as well as *EGFR* gene amplification and mutation with clinical drug response in order to investigate EGFR-mediated resistance.

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*Key Words:* Chemotherapy, oncogene, pharmacogenomics.

## Materials and Methods

*Cell lines and compounds.* The panel of 60 human tumor cell lines of the Developmental Therapeutics Program of the National Cancer Institute (NCI, USA) consisted of leukemia, melanoma, non-small cell lung cancer, colon cancer, renal cancer, ovarian cancer, breast cancer, and prostate carcinoma cells as well as tumor cells of the

central nervous system. Their origin and processing have been described elsewhere (19). We selected a library of 83 standard anticancer drugs taken from the NCI website (http://dtp.nci.nih.gov), in order to obtain an overview of the relevance of EGFR for all clinically relevant compounds. Cytotoxicity of tested compounds towards cells were assayed by the means of sulforhodamine B (SRB) assay (20). All compounds were tested in the range from $10^{-4}$ to $10^{-8}$ M.

*Statistical analysis*. Data from mRNA microarrays of the NCI tumor cell line panel (21,22) were sourced through the NCI website (http://dtp.nci.nih.gov). The Pearson correlation test was used to test the correlation of $\log_{10}IC_{50}$ values with the relative mRNA or protein expression values, as well as the gene copy numbers and the mutational status. This test was implemented using the WinSTAT Program (Kalmia, Cambridge, MA, USA). *p*-Values were calculated for statistical significance, and r-values were calculated for the correlation coefficient. Significant positive correlations ($p<0.05$ and $r>0.2$) between drug $\log_{10}IC_{50}$ and EGFR data indicated that EGFR was a factor for resistance to the specific drug, while significant negative correlations ($p<0.05$ and $r<-0.2$) indicated that EGFR mediated sensitivity to the drug.

## Results

*Correlation of $log_{10}IC_{50}$ for standard anticancer drugs to EGFR expression and mutations*. Dose–response curves of a total of 83 standard anticancer agents were used to calculate $\log_{10}IC_{50}$ values for 60 cell lines of different tumor origin. These values were deposited at the website of the Developmental Therapeutics Program of the NCI (http://dtp.cancer.gov). Data for *EGFR* mRNA and protein expression, as well as *EGFR* gene amplification and mutation, were sourced from the same database. We correlated the $\log_{10}IC_{50}$ values with the EGFR data by Pearson correlation test (Table I). The significant relationship of high $\log_{10}IC_{50}$ values for busulfan to high *EGFR* mRNA ($p=0.019$, $r=0.276$) and protein expression ($p<0.001$, $r=0.444$) of tumor cell lines indicated that *EGFR* expression may represent a factor of resistance to this drug (Table I). However, no significant *p*-values were observed for the correlation between $\log_{10}IC_{50}$ values for busulfan and *EGFR* gene copy number or point mutations, indicating that mRNA or protein expression, but not genetic aberrations in the *EGFR* gene, may affect responsiveness of tumor cells to this drug. The *EGFR* mRNA expression as determined by microarray expression (8/16=50%) or RNase protection assay (11/16=69%) was significantly correlated with alkylating and platinum drugs. Concerning protein expression measured by western blotting, the fraction of drugs with significant correlations was even higher (13/16=81%). *EGFR* gene amplification and mutations were less frequently associated with resistance to alkylating or platinum drugs (2/16=13% and 0/16=0%, respectively).

We also investigated the relationships between EGFR expression or genetic aberrations and drugs of other pharmacological classes. Cellular response to alkylating agents/platinum drugs, anthracyclines/DNA topoisomerase (TOPO) II inhibitors, epipodophyllotoxins/TOPO II inhibitors or anti-hormones frequently correlated with EGFR as determined by *EGFR* RNase protection assay and EGFR western blotting (both frequencies >50%), while cellular response to anti-metabolites, antibiotics and mammalian target of rapamycin (mTOR) inhibitors was less frequently associated with EGFR expression (both frequencies ≤50%). Moderate correlations were found for drugs from the classes of mitotic spindle poisons, camptothecins/TOPO I inhibitors, tyrosine kinase inhibitors, epigenetic inhibitors and the other drug classes investigated. Interestingly, gene copy number or point mutations were considerably less significantly associated with the response of the tumor cell lines to drugs from all pharmacological classes, indicating that genetic alterations are not reliable determinants of drug resistance in this panel of tumor cell lines. EGFR protein expression rather than other measurements was most frequently associated with drug response (47/83=57%) compared to 41% for microarray, 53% for RNase protection, 16% for gene copy number and 8% for gene mutation.

*Correlation of $log_{10}IC_{50}$ for natural products with EGFR expression and mutations*. As a further step, we correlated the top 10 cytotoxic natural products from a database of more than 1,000 phytochemicals derived from medicinal plants. The information for these 10 natural products have already been reported by us (23). The mean $\log_{10}IC_{50}$ values of these 10 compounds across the 60 cell lines of the NCI panel ranged from −8.263(±0.427) to −12.346(±9.44) M (23). We correlated all $\log_{10}IC_{50}$ values for the 10 compounds with EGFR data of the cell lines. As shown in Table I, the cytotoxicity of 6/10 compounds was neither associated with *EGFR* mRNA nor EGFR protein expression, indicating the potential of these natural products in overcoming EGFR-mediated resistance to standard anticancer drugs.

## Discussion

EGFR is well-known as oncogenic factor. The development of specific small molecules and antibodies targeting EGFR represents an attractive treatment strategy to eliminate EGFR-overexpressing tumors (24, 25). It is also long known from transfection experiments that the *EGFR* gene and other human epidermal growth factor receptor family members confer resistance to chemo- and radiotherapy (26-28).

Despite these interesting results, the relevance of EGFR as a factor of resistance to multiple drugs remains ambiguous. In the present investigation, we explored whether EGFR data were correlated with a single drug, a specific drug class, or a broad spectrum of drugs, which might be comparable to the multidrug-resistance phenotype mediated by ATP-binding cassette (ABC) transporters.

Table I. *Correlation of the epidermal growth factor receptor (EGFR) mRNA and protein levels to the log-transformed half-maximal inhibitory concentration ($log_{10}IC_{50}$) data for standard anticancer drugs and natural products in the panel of tumor cell lines.*

| Drug class | | | *EGFR* | | | | |
|---|---|---|---|---|---|---|---|
| | | Pearson correlation | Microarray hybridization | RNase protection assay | Western blotting | Gene copy number | Point mutations |
| | *EGFR* RNase protection assay | *r* | **0.727** | | **0.636** | | |
| | | *p* | **$1.09\times10^{-10}$** | | **$3.98\times10^{-8}$** | | |
| | EGFR western blotting | *r* | **0.609** | **0.636** | | | |
| | | *p* | **$1.99\times10^{-7}$** | **$3.98\times10^{-8}$** | | | |
| Alkylating agents and platinum-based compounds | Busulfan | *r* | **0.276** | **0.339** | **0.444** | 0.090 | –0.032 |
| | | *p* | **0.019** | **0.005** | **$2.41\times10^{-4}$** | 0.250 | 0.407 |
| | Dacarbazine | *r* | **0.263** | **0.300** | **0.287** | 0.123 | $4.64\times10^{-4}$ |
| | | *p* | **0.025** | **0.012** | **0.014** | 0.179 | 0.499 |
| | Thiotepa | *r* | 0.177 | 0.134 | **0.315** | –0.0336 | 0.150 |
| | | *p* | 0.092 | 0.157 | **0.007** | 0.340 | 0.128 |
| | Carmustine | *r* | **0.328** | **0.385** | **0.518** | 0.080 | 0.003 |
| | | *p* | **0.006** | **0.001** | **$1.13\times10^{-5}$** | 0.272 | 0.491 |
| | Lomustine | *r* | 0.057 | **0.237** | 0.102 | **0.260** | 0.056 |
| | | *p* | 0.336 | **0.038** | 0.221 | **0.023** | 0.338 |
| | Semustine | *r* | **0.243** | **0.309** | **0.497** | 0.047 | 0.001 |
| | | *p* | **0.034** | **0.010** | **$3.18\times10^{-5}$** | 0.363 | 0.496 |
| | Bendamustine | *r* | 0.045 | **0.222** | **0.287** | –0.012 | 0.108 |
| | | *p* | 0.368 | **0.048** | **0.014** | 0.464 | 0.209 |
| | Streptozocin | *r* | –0.052 | 0.148 | **0.322** | –0.024 | –0.013 |
| | | *p* | 0.352 | 0.136 | **0.006** | 0.430 | 0.463 |
| | Chlorambucil | *r* | **0.219** | **0.231** | **0.429** | –0.060 | 0.106 |
| | | *p* | **0.049** | **0.040** | **$3.17\times10^{-4}$** | 0.326 | 0.213 |
| | Ifosfamide | *r* | **0.256** | **0.236** | **0.447** | 0.020 | –0.066 |
| | | *p* | **0.027** | **0.039** | **$1.96\times10^{-4}$** | 0.439 | 0.311 |
| | Mafosfamide | *r* | 0.080 | 0.029 | **0.249** | –0.082 | –0.039 |
| | | *p* | 0.276 | 0.413 | **0.027** | 0.268 | 0.386 |
| | Melphalan | *r* | **0.297** | **0.343** | **0.446** | 0.048 | 0.133 |
| | | *p* | **0.012** | **0.005** | **$1.98\times10^{-4}$** | 0.359 | 0.161 |
| | Azathioprine | *r* | –0.045 | 0.147 | 0.176 | **0.246** | 0.053 |
| | | *p* | 0.368 | 0.140 | 0.093 | **0.031** | 0.346 |
| | Carboplatin | *r* | 0.216 | **0.233** | 0.195 | 0.135 | 0.112 |
| | | *p* | 0.052 | **0.034** | 0.068 | 0.152 | 0.199 |
| | Cisplatin | *r* | 0.208 | 0.123 | **0.240** | –0.002 | 0.069 |
| | | *p* | 0.058 | 0.166 | **0.032** | 0.495 | 0.302 |
| | Oxaliplatin | *r* | **0.220** | **0.253** | **0.345** | 0.209 | –0.130 |
| | | *p* | **0.048** | **0.029** | **0.004** | 0.056 | 0.163 |
| | Resistant, n/N | | 8/16 (50%) | 11/16 (69%) | 13/16 (81%) | 2/16 (13%) | 0/16 (0%) |
| | Sensitive, n/N | | 0/16 (0%) | 0/16 (0%) | 0/16 (0%) | 0/16 (0%) | 0/16 (0%) |
| Anti-metabolites | Methotrexate | *r* | 0.201 | 0.195 | 0.191 | 0.066 | 0.072 |
| | | *p* | 0.065 | 0.071 | 0.072 | 0.307 | 0.295 |
| | Trimetrexate | *r* | **0.298** | **0.248** | **0.267** | 0.064 | –0.012 |
| | | *p* | **0.012** | **0.030** | **0.020** | 0.314 | 0.464 |
| | Pemetrexed | *r* | –0.092 | –0.024 | 0.123 | 0.038 | 0.078 |
| | | *p* | 0.250 | 0.430 | 0.178 | 0.388 | 0.281 |
| | Hydroxyurea | *r* | 0.003 | 0.137 | **0.262** | –0.013 | 0.083 |
| | | *p* | 0.491 | 0.155 | **0.023** | 0.462 | 0.268 |
| | Cladribine | *r* | –0.062 | 0.005 | 0.114 | 0.043 | 0.202 |
| | | *p* | 0.321 | 0.485 | 0.195 | 0.374 | 0.063 |
| | Clofarabine | *r* | –0.121 | –0.073 | 0.072 | 0.082 | **0.229** |
| | | *p* | 0.184 | 0.294 | 0.294 | 0.268 | **0.041** |
| | Fludarabine | *r* | –0.041 | 0.059 | 0.007 | 0.050 | 0.049 |
| | | *p* | 0.383 | 0.333 | 0.480 | 0.354 | 0.359 |
| | Mercaptopurine | *r* | 0.180 | **0.253** | 0.180 | **0.311** | **0.311** |
| | | *p* | 0.088 | **0.028** | 0.084 | **0.008** | **0.008** |

Table I. *Continued*

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| Drug class | | | *EGFR* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Pearson correlation | Microarray hybridization | RNase protection assay | Western blotting | Gene copy number | Point mutations |
| | Pentostatin | *r* | 0.078 | 0.010 | **0.236** | –0.065 | 0.088 |
| | | *p* | 0.283 | 0.469 | **0.036** | 0.313 | 0.257 |
| | 6-Thioguanine | *r* | 0.202 | 0.200 | 0.169 | 0.188 | 0.181 |
| | | *p* | 0.064 | 0.066 | 0.098 | 0.075 | 0.085 |
| | Cytarabine | *r* | 0.098 | 0.163 | 0.207 | 0.146 | 0.134 |
| | | *p* | 0.231 | 0.111 | 0.056 | 0.132 | 0.156 |
| | 5-Fluorouracil | *r* | 0.170 | 0.168 | 0.139 | 0.070 | –0.002 |
| | | *p* | 0.100 | 0.103 | 0.145 | 0.297 | 0.495 |
| | Gemcitabine | *r* | 0.035 | 0.046 | **0.228** | 0.064 | 0.161 |
| | | *p* | 0.397 | 0.366 | **0.040** | 0.313 | 0.111 |
| | Tegafur | *r* | **0.225** | 0.178 | 0.063 | –0.027 | –0.071 |
| | | *p* | **0.047** | 0.093 | 0.317 | 0.418 | 0.299 |
| | Resistant, n/N | | 2/14 (14%) | 2/14 (14%) | 4/14 (29%) | 1/14 (7%) | 2/14 (14%) |
| | Sensitive, n/N | | 0/14 (0%) | 0/14 (0%) | 0/14 (0%) | 0/14 (0%) | 0/14 (0%) |
| Mitotic spindle poisons | Docetaxel | *r* | 0.206 | **0.270** | 0.075 | 0.197 | 0.029 |
| | | *p* | 0.064 | **0.022** | 0.287 | 0.069 | 0.414 |
| | Paclitaxel | *r* | **0.368** | **0.306** | **0.229** | 0.107 | 0.096 |
| | | *p* | **0.002** | **0.010** | **0.039** | 0.208 | 0.234 |
| | Vinblastine | *r* | **0.286** | **0.344** | 0.208 | 0.183 | –0.018 |
| | | *p* | **0.015** | **0.004** | 0.056 | 0.081 | 0.446 |
| | Vincristine | *r* | 0.106 | 0.151 | 0.115 | 0.115 | 0.149 |
| | | *p* | 0.215 | 0.129 | 0.192 | 0.191 | 0.131 |
| | Resistant, n/N | | 2/4 (50%) | 3/4 (75%) | 1/4 (25%) | 0/5 (0%) | 0/5 (0%) |
| | Sensitive, n/N | | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) |
| Anthracyclines/ TOPO II inhibitors | Daunorubicin | *r* | **0.300** | **0.312** | **0.376** | –0.009 | 0.082 |
| | | *p* | **0.017** | **0.015** | **0.003** | 0.476 | 0.284 |
| | Doxorubicin | *r* | **0.335** | **0.361** | **0.448** | 0.003 | 0.027 |
| | | *p* | **0.005** | **0.003** | **$1.68\times10^{-4}$** | 0.492 | 0.419 |
| | Epirubicin | *r* | **0.283** | **0.334** | **0.370** | –0.003 | 0.038 |
| | | *p* | **0.016** | **0.006** | **0.002** | 0.492 | 0.387 |
| | Idarubicin | *r* | **0.251** | **0.225** | **0.351** | 0.049 | 0.112 |
| | | *p* | **0.031** | **0.048** | **0.003** | 0.358 | 0.204 |
| | Mitoxantrone | *r* | 0.051 | 0.015 | **0.258** | –0.112 | 0.119 |
| | | *p* | 0.353 | 0.454 | **0.023** | 0.197 | 0.185 |
| | Resistant, n/N | | 4/5 (80%) | 4/5 (80%) | 5/5 (100%) | 0/5 (0%) | 0/5 (0%) |
| | Sensitive, n/N | | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) |
| Antibiotics | Bleomycin | *r* | –0.084 | –0.126 | 0.098 | **–0.216** | 0.213 |
| | | *p* | 0.266 | 0.172 | 0.228 | **0.048** | 0.052 |
| | Dactinomycin | *r* | **0.448** | **0.425** | **0.503** | 0.089 | –0.090 |
| | | *p* | **$4.95\times10^{-4}$** | **0.001** | **$7.19\times10^{-5}$** | 0.266 | 0.263 |
| | Mitomycin C | *r* | **0.233** | 0.124 | **0.283** | –0.107 | 0.144 |
| | | *p* | **0.040** | 0.176 | **0.014** | 0.208 | 0.138 |
| | Mithramycin | *r* | **0.426** | 0.167 | 0.160 | –0.0209 | –0.062 |
| | | *p* | **$5.90\times10^{-4}$** | 0.111 | 0.117 | 0.439 | 0.325 |
| | Resistant, n/N | | 3/4 (75%) | 1/4 (25%) | 2/4 (50%) | 0/4 (0%) | 0/4 (0%) |
| | Sensitive, n/N | | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 1/4 (25%) | 0/4 (0%) |
| Epipodophyllotoxins/ TOPO II inhibitors | Etoposide | *r* | 0.190 | **0.313** | **0.322** | 0.087 | 0.061 |
| | | *p* | 0.080 | **0.009** | **0.007** | 0.259 | 0.325 |
| | Teniposide | *r* | **0.262** | **0.294** | **0.476** | 0.031 | 0.076 |
| | | *p* | **0.025** | **0.013** | **$7.01\times10^{-5}$** | 0.407 | 0.285 |
| | m-AMSA, amsacrine | *r* | 0.115 | 0.097 | **0.242** | –0.086 | 0.127 |
| | | *p* | 0.195 | 0.233 | **0.031** | 0.256 | 0.169 |
| | Resistant, n/N | | 1/3 (33%) | 2/3 (66%) | 3/3 (100%) | 0/4 (0%) | 0/4 (0%) |
| | Sensitive, n/N | | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) |
| Camptothecins/ TOPO I inhibitors | Camptothecin | *r* | 0.089 | 0.066 | **0.374** | –0.084 | 0.191 |
| | | *p* | 0.268 | 0.324 | **0.003** | 0.277 | 0.089 |

Table I. *Continued*

Table I. *Continued*

| Drug class | | | *EGFR* | | | | |
|---|---|---|---|---|---|---|---|
| | | Pearson correlation | Microarray hybridization | RNase protection assay | Western blotting | Gene copy number | Point mutations |
| | Irinotecan | *r* | 0.078 | **0.281** | 0.201 | –0.091 | 0.011 |
| | | *p* | 0.281 | **0.018** | 0.066 | 0.250 | 0.467 |
| | Topotecan | *r* | 0.148 | 0.067 | **0.308** | –0.023 | **0.244** |
| | | *p* | 0.134 | 0.309 | **0.008** | 0.431 | **0.031** |
| | Resistant, n/N | | 0/3 (0%) | 1/3 (33%) | 2/3 (66%) | 0/3 (0%) | 1/3 (33%) |
| | Sensitive, n/N | | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) |
| Antihormones | Fulvestrant | *r* | 0.199 | **0.332** | **0.361** | 0.162 | –0.140 |
| | | *p* | 0.068 | **0.006** | **0.003** | 0.113 | 0.147 |
| | Tamoxifen | *r* | **0.349** | **0.513** | **0.545** | **0.226** | –0.042 |
| | | *p* | **0.005** | **$2.27\times10^{-5}$** | **$4.01\times10^{-6}$** | **0.042** | 0.377 |
| | Toremifen | *r* | 0.117 | **0.299** | **0.381** | 0.091 | –0.085 |
| | | *p* | 0.199 | **0.015** | **0.002** | 0.254 | 0.270 |
| | Anastrozol | *r* | –0.128 | 0.073 | –0.044 | –0.058 | 0.006 |
| | | *p* | 0.191 | 0.314 | 0.382 | 0.346 | 0.484 |
| | Exemestane | *r* | **0.298** | **0.237** | 0.068 | 0.026 | 0.055 |
| | | *p* | **0.011** | **0.039** | 0.307 | 0.425 | 0.342 |
| | Megostrol | *r* | 0.036 | 0.202 | **0.239** | –0.049 | **–0.237** |
| | | *p* | 0.396 | 0.068 | **0.036** | 0.357 | **0.037** |
| | Raloxifene | *r* | **0.293** | **0.535** | **0.500** | **0.288** | –0.119 |
| | | *p* | **0.013** | **$0.15\times10^{-6}$** | **$2.81\times10^{-5}$** | **0.013** | 0.184 |
| | Resistant, n/N | | 3/7 (43%) | 5/7 (71%) | 5/7 (71%) | 2/7 (29%) | 0/7 (0%) |
| | Sensitive, n/N | | 0/7 (0%) | 0/7 (0%) | 0/7 (0%) | 0/7 (0%) | 1/7 (14%) |
| Tyrosine kinase inhibitors | Axitinib | *r* | –0.151 | 0.088 | 0.155 | –0.028 | –0.011 |
| | | *p* | 0.145 | 0.271 | 0.136 | 0.423 | 0.470 |
| | Crizotinib | *r* | **0.344** | **0.464** | **0.408** | 0.151 | 0.030 |
| | | *p* | **0.004** | **$1.41\times10^{-4}$** | **$6.79\times10^{-4}$** | 0.127 | 0.412 |
| | Dasatinib | *r* | **–0.284** | **–0.489** | **–0.237** | –0.126 | 0.206 |
| | | *p* | **0.021** | **$1.34\times10^{-4}$** | **0.043** | 0.184 | 0.069 |
| | Erlotinib | *r* | **–0.356** | **–0.450** | **–0.326** | **–0.245** | 0.164 |
| | | *p* | **0.003** | **$1.94\times10^{-4}$** | **0.006** | **0.029** | 0.107 |
| | Gamitrinib G-TPP | *r* | **0.442** | **0.503** | **0.315** | 0.060 | –0.150 |
| | | *p* | **$2.56\times10^{-4}$** | **$3.28\times10^{-5}$** | **0.008** | 0.327 | 0.129 |
| | Gamitrinib G4 | *r* | **0.487** | **0.341** | **0.282** | 0.118 | –0.044 |
| | | *p* | **$5.20\times10^{-5}$** | **0.005** | **0.015** | 0.187 | 0.370 |
| | Gefitinib | *r* | –0.090 | –0.128 | –0.124 | –0.132 | **–0.236** |
| | | *p* | 0.250 | 0.173 | 0.174 | 0.159 | **0.036** |
| | Imatinib | *r* | 0.181 | **0.343** | **0.263** | 0.181 | –0.074 |
| | | *p* | 0.099 | **0.007** | **0.029** | 0.097 | 0.299 |
| | Lapatinib | *r* | 0.206 | –0.058 | –0.023 | 0.086 | 0.006 |
| | | *p* | 0.071 | **0.342** | 0.436 | 0.271 | 0.483 |
| | Nilotinib | *r* | **0.242** | **0.342** | 0.205 | **0.430** | –0.071 |
| | | *p* | **0.042** | **0.007** | 0.071 | **$6.67\times10^{-4}$** | 0.306 |
| | Sorafenib | *r* | 0.201 | **0.290** | 0.207 | 0.056 | **–0.317** |
| | | *p* | 0.077 | **0.019** | 0.068 | 0.344 | **0.010** |
| | Sunitinib | *r* | 0.129 | **0.267** | 0.143 | 0.120 | –0.070 |
| | | *p* | 0.168 | **0.022** | 0.140 | 0.182 | 0.300 |
| | Vandetanib | *r* | –0.204 | –0.193 | –0.070 | **–0.240** | 0.007 |
| | | *p* | 0.066 | 0.079 | 0.302 | **0.036** | 0.481 |
| | Vemurafenib | *r* | **0.303** | **0.29** | 0.153 | 0.163 | **–0.274** |
| | | *p* | **0.010** | **0.014** | 0.124 | 0.108 | **0.018** |
| | Resistant, n/N | | 5/14 (36%) | 8/14 (57%) | 4/14 (29%) | 1/14 (7%) | 0/14 (0%) |
| | Sensitive, n/N | | 2/14 (14%) | 2/14 (14%) | 2/14 (14%) | 2/14 (14%) | 3/14 (21%) |
| mTOR inhibitors | Everolimus | *r* | –0.190 | –0.169 | –0.185 | –0.139 | 0.168 |
| | | *p* | 0.076 | 0.104 | 0.080 | 0.147 | 0.101 |
| | Temsirolimus | *r* | 0.081 | 0.058 | 0.107 | –0.133 | –0.027 |

Table I. *Continued*

Table I. *Continued*

| Drug class | | | *EGFR* | | | | |
|---|---|---|---|---|---|---|---|
| | | Pearson correlation | Microarray hybridization | RNase protection assay | Western blotting | Gene copy number | Point mutations |
| | | *p* | 0.278 | 0.336 | 0.212 | 0.160 | 0.421 |
| | Sirolimus | *r* | –0.027 | –0.071 | –0.005 | **–0.227** | –0.023 |
| | | *p* | 0.420 | 0.299 | 0.486 | **0.042** | 0.431 |
| | Resistant, n/N | | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) |
| | Sensitive, n/N | | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 1/3 (33%) | 0/3 (0%) |
| Epigenetic inhibitors | Azacytidine | *r* | 0.140 | 0.096 | 0.207 | 0.146 | –0.073 |
| | | *p* | 0.150 | 0.239 | 0.058 | –0.086 | 0.084 |
| | 3-Deazacytidine | *r* | –0.065 | 0.001 | –0.076 | **0.331** | 0.100 |
| | | *p* | 0.346 | 0.497 | 0.322 | **0.005** | 0.227 |
| | Decitabine | *r* | 0.090 | **0.241** | –0.027 | **0.232** | –0.018 |
| | | *p* | 0.253 | **0.035** | 0.419 | **0.040** | 0.447 |
| | Dihydro-5-azacytidine | *r* | **0.256** | **0.352** | **0.309** | **0.241** | –0.089 |
| | | *p* | **0.029** | **0.004** | **0.009** | **0.033** | 0.254 |
| | Vorinostat | *r* | **0.377** | **0.360** | **0.291** | 0.052 | –0.024 |
| | | *p* | **0.002** | **0.003** | **0.013** | 0.348 | 0.430 |
| | Resistant, n/N | | 2/5 (40%) | 3/5 (60%) | 2/5 (40%) | 3/5 (60%) | 0/5 (0%) |
| | Sensitive, n/N | | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) |
| Other | All-trans-retinol | *r* | 0.048 | 0.067 | **0.261** | –0.073 | 0.037 |
| | | *p* | 0.371 | 0.321 | **0.031** | 0.303 | 0.399 |
| | Asparaginase | *r* | –0.005 | 0.083 | **0.286** | –0.141 | –0.070 |
| | | *p* | 0.485 | 0.269 | **0.014** | 0.144 | 0.300 |
| | Bortezomib | *r* | 0.161 | **0.260** | **0.258** | –0.085 | –0.189 |
| | | *p* | 0.113 | **0.025** | **0.024** | 0.261 | 0.076 |
| | Zoledronate | *r* | **–0.370** | –0.110 | –0.150 | –0.160 | –0.155 |
| | | *p* | **0.002** | 0.208 | 0.132 | 0.114 | 0.121 |
| | Estramustine | *r* | **0.345** | **0.438** | **0.415** | 0.164 | 0.057 |
| | | *p* | **0.004** | **$3.33\times10^{-4}$** | **$5.47\times10^{-4}$** | 0.107 | 0.335 |
| | Resistant, n/N | | 1/5 (20%) | 2/5 (40%) | 4/5 (80%) | 0/5 (0%) | 0/5 (0%) |
| | Sensitive, n/N | | 1/5 (20%) | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) | 0/5 (0%) |
| Grand total | Resistant, n/N | | 31/83 (37%) | 42//83 (51%) | 45/83 (54%) | 9/83 (11%) | 3/83 (4%) |
| | Sensitive, n/N | | 3/83 (4%) | 2/83 (2%) | 2/83 (2%) | 4/83 (5%) | 4/83 (5%) |
| | Significant associations, n N | | 34/83 (41%) | 44/83 (53%) | 47/83 (57%) | 13/83 (16%) | 7/83 (8%) |
| Natural products | Maytansine | *r* | 0.055 | 0.153 | 0.234 | NA | NA |
| | | *p* | 0.360 | 0.157 | 0.057 | NA | NA |
| | Hellebrin | *r* | 0.143 | –0.109 | 0.088 | NA | NA |
| | | *p* | 0.193 | 0.254 | 0.294 | NA | NA |
| | Glycoside | *r* | 0.067 | –0.059 | –0.039 | NA | NA |
| | | *p* | 0.321 | 0.339 | 0.392 | NA | NA |
| | Stilbene derivative 30 | *r* | 0.133 | –0.030 | 0.157 | NA | NA |
| | | *p* | 0.210 | 0.429 | 0.167 | NA | NA |
| | Stilbene derivative 12 | *r* | 0.118 | 0.162 | 0.163 | NA | NA |
| | | *p* | 0.238 | 0.162 | 0.154 | NA | NA |
| | Quassinoid | *r* | **0.235** | **0.257** | 0.149 | NA | NA |
| | | *p* | **0.039** | **0.027** | 0.130 | NA | NA |
| | Stilbene derivative 18 | *r* | **0.314** | 0.163 | 0.225 | NA | NA |
| | | *p* | **0.027** | 0.164 | 0.081 | NA | NA |
| | Proscillaridin | *r* | **0.225** | 0.191 | 0.126 | NA | NA |
| | | *p* | **0.045** | 0.075 | 0.168 | NA | NA |
| | Peltatin | *r* | –0.081 | –0.054 | –0.092 | NA | NA |
| | | *p* | 0.283 | 0.350 | 0.255 | NA | NA |
| | Bruceantin | *r* | **0.427** | **0.447** | **0.276** | NA | NA |
| | | *p* | **$4.15\times10^{-4}$** | **$2.21\times10^{-4}$** | **0.016** | NA | NA |
| | Resistant, n/N | | 4/10 (40%) | 2/10 (20%) | 1/10 (10%) | | |
| | Sensitive, n/N | | 0/10 (0%) | 0/10 (0%) | 0/10 (0%) | | |

Bold numbers: Significant associations of between $\log_{10}IC_{50}$ and EGFR ($p<0.05$). $r>0.2$ demonstrated a positive association which implied EGFR mediated resistance and *vice versa*.

Our results showed that the expression of *EGFR* mRNA or EGFR protein was mainly correlated with the $\log_{10}IC_{50}$ of anti-hormones and inducers of DNA damage, *e.g.* alkylating agents/platinum drugs, anthracyclines/TOPO II inhibitors, epipodophyllotoxins/TOPO II inhibitors, but rarely with anti-metabolites, antibiotics and mTOR inhibitors. By comparison, cross-resistance mediated by P-glycoprotein comprises the drug classes of anthracyclines/ TOPO II inhibitors, mitotic spindle poisons and some antibiotics, but not alkylating agents/platinum drugs or anti-hormones (29). Hence cross-resistance mediated by P-glycoprotein and EGFR exhibits overlapping but not identical profiles. It has been reported that EGFR regulates resistance to various modes of cell death (*e.g.* apoptosis, autophagy, and anoikis) (30-32). The suppression of cell death represents an imperative step in carcinogenesis but also in resistance to chemotherapy. EGFR protein expression was shown to mediate resistance to cisplatin in the clinic by Aichler *et al*. (33). Sensitivity to erlotinib was reported in a clinical trial by Juergens *et al*. (34) and in preclinical studies by Quesnelle *et al*. (35). These results are consistent with our *in vitro* results with the NCI60 cell line panel.

Another result of our study was that drug resistance of the NCI panel of cell lines correlated with *EGFR* mRNA and protein expression but not with gene copy number or gene mutations. This may be surprising at first sight, since many investigations reported that *EGFR* gene amplification and mutations are associated with treatment failure and poor prognosis (36-39). However, comparable results were observed by others for the NCI cell line panel showing that response did not correlate with *EGFR* amplification but with *EGFR* gene expression (40). Still, it cannot be excluded that the current panel of tumor cell lines, which consists of diverse tumor types (carcinoma of colon, lung, kidney, prostate, ovarian and breast, CNS tumors, melanoma, leukemia) was too heterogeneous to allow elucidation of significant relationships between the mutational status and drug resistance.

In addition to standard anticancer drugs, we investigated phytochemicals. Previously, we described a library of more than 1,000 chemically characterized compounds derived from medicinal plants used in Chinese medicine (23). We tested correlations of the $\log_{10}IC_{50}$ for the 10 most cytotoxic natural products and the EGFR parameters and found that for the majority of these phytochemicals (6/10), there was no significant correlation with any of the EGFR data. Such compounds can be further developed as drugs with activity against EGFR-overexpressing or mutated tumors, as they were not part of the EGFR-associated cross-resistance profile.

In conclusion, our study revealed the association between EGFR and resistance to a broad range of clinical drugs. EGFR is not only a factor in carcinogenesis, but probably also plays an essential role in development of drug resistance.

## Conflicts of Interest

The Authors declare that there is no conflict of interest.

## Authors' Contributions

Ge Yan contributed to article composing. Dr. Thomas Efferth contributed to the data generation and revision.

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