Review

The COXEN Principle: Translating Signatures of In vitro Chemosensitivity into Tools for Clinical Outcome Prediction and Drug Discovery in Cancer

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Abstract

Substantial effort has been devoted to in vitro testing of candidate chemotherapeutic agents. In particular, the United States National Cancer Institute Developmental Therapeutics Program (NCI-DTP) Human Tumor Cell Line Screen has screened hundreds of thousands of compounds and extracts, for which data on more than 40,000 compounds tested on a panel of 60 cancer cell lines (NCI-60) are publically available. In tandem, gene expression profiling has brought about a sea change in our understanding of cancer biology, allowing discovery of biomarkers or signatures able to characterize, classify, and prognosticate clinical behavior of human tumors. Recent studies have used tumor profiling matched to clinical trial outcome data to derive gene expression models predicting therapeutic outcomes, though such efforts are costly, time-consuming, tumor type-specific, and not amenable to rare diseases. Furthermore, addition of new or established drugs to multidevelopment of these models may be possible and raise important implications for future trial design and drug discovery. Cancer Res; 70(5); 1753–8. ©2010 AACR.

Introduction

Few technological innovations have transformed biology to the extent high throughput molecular profiling has done. Such efforts, coupled with a wide variety of platforms for statistical and biological interpretation as well as interinstitutional sharing of the data produced by these assays, have changed how we study neoplasia. Recent advances suggest that these technologies may change the way pathologists and oncologists approach the diagnosis and nosology of tumors, and key recent reports suggest that the use of prediction frameworks developed from gene expression profiling data, roughly, gene expression models (GEM), can be developed and clinically implemented to provide accurate prognostication of disease course (1) and even therapeutic benefit (2). For example, in bladder cancer, studies have developed GEMs predicting chemotherapeutic response of muscle invasive tumors (3, 4).

Although these efforts lay the foundation for implementation of molecular tools to predict patient benefit from standard cancer chemotherapeutic regimens, they come with important limitations. Such GEMs must be developed a posteriori, on the basis of profiling patient tumors at baseline followed by supervised biomarker discovery comparing clinical responders and nonresponders, with subsequent validation in independent trials. Thus, such strategies are not adaptable to rare orphan tumors (insufficient numbers for trials) or cancers of unknown primary (among the 10 most common cancer diagnoses; ref. 5). Such strategies also do not suggest a “salvage” therapeutic option for predicted nonresponders, and such approaches are not adaptable for a priori stratification of patients for novel agents in which clinical trial outcome is not known. Finally, upon addition of approved or investigational agents to standard combination regimens, existing GEMs must perforce be rebuilt and prospectively revalidated.

The United States National Cancer Institute’s Developmental Therapeutics Program’s (NCI-DTP) NCI-60 Human Tumor Cell Line Screen, which has tested 60 cancer cell lines derived from 9 common histologies tested with more than 110,000 compounds of which more than 45,000 are publicly available, provides a rich database of in vitro drug response data (6). Initially intended as a government-sponsored drug discovery pipeline, this initiative has already made significant contributions directly to this objective. In addition, these data are a rich source of information that could be mined for additional biological insights. For example, reports as early as 2001 showed that using gene expression profiling of these 60 cell lines, coupled to the vast in vitro response data in the NCI-60 screen, investigators could develop signatures predictive...
of sensitivity within the same cell line panel (7). Taken a step further, what if the language of gene expression could be used to systematically extrapolate drug sensitivity results observed in cell culture screening to predict tumor behavior in patients? Surprisingly, only lately has this been shown by us (8, 9) and by others (10).

Motivation for the Development of the COXEN Algorithm

Bladder cancer–derived cell lines were not included in the NCI-60 cell line panel. Our desire to develop chemotherapeutic response prediction models for this tumor type prompted us to develop a collection of nearly 40 commonly used bladder cancer cell lines, which we called BLA-40. These were profiled for their baseline gene expression using oligonucleotide microarrays, and tested in vitro for sensitivity to several chemotherapeutic drugs relevant in the treatment of urothelial cancer, including gemcitabine, cisplatin, and paclitaxel. Using a classification algorithm that favors discovery of robust, parsimonious gene expression models and is relatively resistant to “overfitting” (11), we were able to show in cross-validation studies correct prediction of drug sensitivity across the three drugs. Most compellingly, given the frequent use of doublet (gemcitabine-cisplatin) therapy for muscle invasive bladder cancer (12), we were able to predict response to doublet combination chemotherapy within the cell lines with 80% accuracy (P = 0.0002; ref. 13). We recently reported a similar effort for the dual EGFR-HER2 inhibitor, lapatinib (14).

With this expression-profiled bladder cancer cell panel in hand but lacking the resources to carry out large-scale drug screening, we formulated the hypothesis that perhaps clustering of the NCI-60 gene expression data with that of BLA-40 would allow us to project the in vitro drug sensitivity data available on the NCI-60 to the bladder cancer cell lines. Unfortunately, this simplistic approach was not successful as the cell lines clustered primarily by histological subtype. To correct for this, we first identified the genes whose expression in the NCI-60 was related to drug sensitivity and then determined which of these genes maintained concordant expression in the BLA-40 panel. This is done through comparisons of correlation matrices. For example, for a list of 50 candidate sensitivity genes, a 50 × 50 matrix of the correlation of expression of the 50 genes, across the first cell line data set, to each of the other 50 genes is generated. The same matrix is then prepared from the second cell line data set gene expression data. Finally, each row (i.e., each gene-candidate biomarker) of these two correlation matrices is then correlated between the two matrices to generate a second correlation coefficient (the COXEN coefficient), reflective of whether that particular candidate biomarker maintains concordant relationships to the other candidate genes. A correlation coefficient cutoff may thus be used to select the concordant subset, which are then used for GEM development. Thus, this “correlation of correlations” step allows selection, through requiring intercohort concordant expression, of genes relevant to both cell line panels while maintaining strict independence of the data sets with respects to the tumor drug sensitivity outcomes in the second cohort. We call this process COXExpression Extrapolation, or COXEN, and have employed it for several combinations of cell line panel to cell line panel and cell line panel to human tumor projections as described below. COXEN was also used for computational drug discovery in bladder cancer using in vitro sensitivity data obtained from the NCI-60 panel. These applications are summarized in Fig. 1.

Applications of COXEN in Therapeutic Response Prediction

Our first report of the development and use of this methodology described several applications of COXEN (8). The first was to use gene expression data for the NCI-60 cell lines, coupled with their published in vitro sensitivity data to the drugs cisplatin and paclitaxel, to predict sensitivity of our BLA-40 cell lines. By using a COXEN step to select concordant genes from NCI-60 data-derived candidate sensitivity biomarkers and implement them into GEMs, we were able to predict sensitive and resistant bladder cancer cell lines with accuracies of 85% for cisplatin and 78% for paclitaxel on the basis of the NCI-60 cell line data alone.

The next application used gene expression and in vitro sensitivity data for paclitaxel and tamoxifen to predict actual therapeutic outcomes in two clinical studies in which breast cancer patients had their tumors expression profiled. In the first case COXEN used paclitaxel in vitro cell line data to generate GEMs, which were applied to the clinical trial response data for the related taxane, docetaxel. Three such models exhibited a classification accuracy of 75% for the 11 responders and 13 nonresponders in this trial. A second clinical study–based implementation used a similar approach to predict metastatic recurrence of 60 estrogen receptor positive breast cancer patients treated with tamoxifen whose tumor tissues were gene expression profiled before therapy. Based on GEMs derived from concordant subsets of NCI-60 sensitivity data and untreated human breast tumors derived biomarkers, we were able to predict with 71% accuracy recurrence in this patient cohort (8).

Recently, we have undertaken an extensive validation of the COXEN methodology using data from an additional seven independent clinical trials of nearly 500 patients of diverse geographic and ethnic backgrounds (9). Importantly, 233 of these patients were from trials with prospective enrollment. We developed drug-specific GEMs, again, by comparing gene expression in cell lines from the NCI-60 screen on the basis of in vitro sensitivity and used separate breast, bladder, and ovarian cancer gene expression data sets (without response data and unrelated to those trials examined in the study) for the COXEN step. Then, concordant sensitivity biomarkers were used to develop COXEN GEMs, which were evaluated singly and in combination for ability to predict response and survival outcomes for several published studies including bladder cancer patients (N = 59) treated with methotrexate,
vinblastine, doxorubicin, and cisplatin (MVAC), breast cancer patients \( (N = 275) \) treated with 5-fluorouracil, doxorubicin, and cyclophosphamide, and ovarian cancer patients \( (N = 143) \) treated with combination platinum-based therapies. Importantly, for two studies of bladder cancer tested, these COXEN GEMs did well for patients in both the neoadjuvant \( (N = 45; \text{refs. 3, 15}) \) and advanced tumor \( (N = 14; \text{ref. 4}) \) settings. When single drug GEMs for each of MVAC were combined, prediction scores found to be significantly different between responders and nonresponders \( (P = 0.002 \text{ and } 0.03, \text{respectively}) \), resulting in a sensitivity of 83%, specificity 64%, positive predictive value of 71%, and negative predictive value of 78%. Compellingly, in the neoadjuvant data set \( (3, 15) \), which was large enough for multivariate analysis, the COXEN GEM scores were found to be independent of a variety of traditional clinicopathologic parameters and the only parameter independently associated with response.

For breast and ovarian cancer, the COXEN GEMs developed from NCI-60 cell line testing was done similarly. Where single drug GEMs for doxorubicin, cyclophosphamide, and 5-fluorouracil did well in two separate gene expression–profiled breast cancer drug outcome studies \( (N = 133 \text{ and } N = 45, \text{respectively}) \), combination GEMs for each of these drugs—used in both studies—were significantly associated with patient responses and survival status (as were the outcomes studied, respectively). This finding was associated with a sensitivity of 71% and specificity of 53%. In a third gene expression–matched neoadjuvant study of gemcitabine, epirubicin, and docetaxel for breast cancer patients \( (N = 100) \), each single-drug COXEN GEM, as well as a three-drug combination GEM, provided prediction scores significantly different between responders and nonresponders. In the first trial, the only case in which complete clinical data were available for multivariate analyses, both estrogen receptor status and the combination GEM were found to be significantly independently prognostic for response. Similarly, in two studies of ovarian cancer (adjuvant \( N = 119 \), neoadjuvant \( N = 24) \), COXEN GEMs for carboplatin and paclitaxel were both significantly predictive of response and survival, as well as independently predictive of response to therapy in the
larger adjuvant trial in which complete clinicopathologic data were available for multivariate analysis.

One interesting aspect of these findings that pertains to the interplatform and interhistology ability of COXEN was illustrated by a recent analysis we subsequently did on the breast and ovarian cancer studies from the above report. Wishing to determine whether cell line training set composition (as regards cell line tissue of origin) impacted the accuracy of prediction, we excluded breast cancer (N = 5) and ovarian cancer (N = 7) cell lines from the NCI-60 panel and re-derived GEMs. We found that the GEM predictions derived without breast or ovarian cancer cell lines included in training were essentially unchanged.7 In the same vein, in the Williams and colleagues report, we specifically examined whether the stage composition of the data set used for the COXEN step (specifically, whether the tumors did or did not exhibit muscle invasion) changed the results. We found that either way it resulted in essentially identical outcome predictions (9).

Applications of COXEN in Computational Drug Screening

We also reported an application of COXEN for in silico drug discovery. The publically available NCI-60 gene expression profiling, was used to develop drug-specific candidate biomarkers, concordant between the NCI-60 and BLA-40, which were implemented as GEMs predictive of sensitivity in the bladder cell lines in vitro. In fact, 139 compounds were identified for which >35% of the BLA-40 were predicted to be sensitive, of which 8 had >50% predicted sensitive. The top hit for this screen was the cytotoxic imidazoacridinone, NSC-637993, which we showed empirically to exhibit submicromolar GI50 in >60% of the BLA-40 cells. In comparison, the anchor drug of bladder cancer combination chemotherapy, cisplatin, exhibits submicromolar GI50 in only 22% of cells (8). Perhaps more importantly, we also found that a structurally similar compound, C1311, was also a top COXEN hit. This finding is relevant because: (1) COXEN computational screening was able to find closely related analogs; (2) C1311 has significant cytotoxic activity in vitro and in vivo for a range of cancer cell lines (16); (3) C1311 has been tested in phase 1 (17 18 19) and phase 2 (20) trials for breast and other advanced solid tumors, potentially facilitating rapid translation of C1311 to bladder cancer patients.

In prior studies the cytotoxic activity of C1311 has been evaluated in 16 human cell lines from solid tumor types and leukemias producing a median IC50 of 0.3 μM (16). In vitro, C1311 caused 77% growth inhibition of HT29 colon cancer xenografts compared with 17% for paclitaxel (21). Importantly, no cross-resistance was seen between in vitro sensitivity to C1311 and doxorubicin or paclitaxel, two agents used in human bladder cancer, opening the possibility of effective combinations of C1311 with these agents. Consistent with these findings, we recently completed testing of C1311 in vitro in our BLA-40 panel and observed a median GI50 of 0.5 μM.5

Questions and Future Directions

The ability of the COXEN methodology to produce, solely on the basis of gene expression profiling coupled to in vitro NCI-60 testing, predictive models capable of stratifying patients is suggestive of several important applications that address limitations of gene expression models derived from a posteriori analysis of gene expression against patient treatment outcomes. Principally, these studies show that a priori stratification of patients is possible, offering the opportunity to substantially reduce the size of clinical trials for novel agents (22), while potentially avoiding treating patients who are unlikely to receive any clinical benefit from investigational or even standard care regimens. For example, in the case of bladder cancer, cisplatin-based combination chemotherapy is associated with substantial morbidity and up to 3% mortality (12). The ability to stratify patients on the basis of probability of response to standard therapies could spare the substantial proportion of therapeutic nonresponders (~50% in larger studies; ref. 12) the toxicity associated with therapy while preventing delay of surgery in the neoadjuvant setting (23). As authors have observed before, for advanced and inoperable patients, earlier triage of predicted nonresponders to investigational agents could take place before further deterioration of performance status, a key factor for survival time in advanced disease (4).

In the case of investigational agents, particularly targeted agents, increasing experience in this area has suggested that a priori stratification of patients is essential to design trials enriched with responders and to exclude participants unlikely to enjoy any clinical benefit (22). Recent a posteriori studies have found that in some cases, particular genetic lesions confer exquisite sensitivity or resistance to targeted agents (24, 25), whereas we note that, in the case of EGFR mutations and drug response in non–small cell lung cancer (NSCLC), between ~5 to 15% of mutation-negative patients still enjoy a therapeutic response (24). We suggest that constructing GEMs a priori on the basis of in vitro response by using COXEN might offer the opportunity to represent a core responder phenotype with gene expression that can be further refined when combined with targeted evaluations such as mutations or activity of signaling intermediates. Supporting this idea is a recent report by Balko and colleagues, who reported signatures of gefitinib sensitivity from in vitro studies that were predictive of EGFR mutation and activation status in NSCLC tissues (26). In addition, there is no reason that predictive nomograms could not be built on the basis of traditional clinicopathologic characteristics, mutation or activation markers, and yet include a GEM; indeed, we have incorporated phosphoprotein biomarkers into the development of combination GEMs for prediction of BLA-40 sensitivity to lapatinib (27).

5 D. Theodorescu and J.K. Lee, unpublished observations.

This technology raises the specter of other possible implementations. For example, future studies in vitro and in animal models might show that COXEN GEMs can predict efficacious agents for nonresponders to standard of care agents. Could such a strategy be implemented to suggest an approved agent or combination for rare orphan tumor histologies, in which standard of care consists of only empiric multi-agent therapies because large randomized trials are not feasible? Most provocatively, could this type of technology be employed to offer other approved anticancer agents to predicted standard of care nonresponders? Similar issues have been raised by other investigators in the field, who have questioned whether prospective validation might always be necessary, particularly in critical cases such as second or third line therapy for treatment failures in which no standard of care exists (28). Although the practical and ethical concerns of these kinds of implementations are manifest and deserve serious consideration and debate, it is clear that novel trial formats will be necessary to evaluate such interventions that are a posteriori outside of current designs. One potential way to address these concerns may be to use methodologies like COXEN GEMs to select therapies to add to standard of care regimens, as is often the trial design used to show efficacy for promising agents. Clearly, though, complex pharmacologic interactions will need to be considered in any combination that is computationally recommended.

From the standpoint of development, two significant opportunities are offered by COXEN. This technology can be used early on to evaluate which tumor histologies are most likely to respond to newly developed agents, suggesting ways to enrich early clinical trials with such patients increasing the chances for success. This approach could also be used in the unfortunate case of failed clinical trials for a drug salvage and/or repositioning strategy in another tumor system, with the theoretical ability to find success in a different tumor histology after trial failures in its primary target. Such trials would be especially efficient and more rapid because the pharmacology and toxicity of the drug would have been already well documented in prior studies.

Finally, whereas we have used COXEN to link cell line panels to other panels or to human tumors by virtue of the common language of gene expression, the principle is not limited by the platform. As such, we are in the process of evaluating the ability of COXEN to generate predictive biomarkers using DNA, microRNA, as well as proteomic data sets from tumors and body fluids.

Caveats and Limitations

Several limitations must be taken into consideration and interpretation of the COXEN findings. First, except for 233 of the breast cancer patients in the most recent report, the patient cohorts in which we have studied the predictive ability of the COXEN GEMs were not prospectively enrolled, which can introduce selection biases. Though this limits the generality of interpretation of the potential role of GEMs in such a setting, it is reassuring to have observed significant prediction, retrospectively, in smaller patient subsets across multiple tumor types reported from geographically different areas. It is revealing, however, that recently this issue has come to the attention of the U.S. Food and Drug Administration, who have suggested in their nonbinding recommendations, Draft Guidance: In vitro Diagnostic Multivariate Index Assays (29), that “use of archived samples and/or retrospective data may sometimes be used to support clearance or approval, provided the study design and sample composition reflect the intended use of the device in the intended population.”

Beyond the larger concerns of how and under what circumstances COXEN or other powerful predictive technologies may be used or approved for use in patients (28), COXEN raises important technical implementation questions. For example, it certainly is not customary in the general practice of oncology for most cancer types to subject patients to the additional morbidity of biopsy of, let alone metastectomy, for presumed metastatic disease. If COXEN GEMs are capable of predicting sensitivity in such a setting, is the original, primary tumor from the definitive resection even the correct lesion to profile for prediction? If not, what degree of efficacy, however defined, of predictive GEMs must be shown to outweigh the risk of invasive surgical or imaging-guided sampling procedures (as has been reported recently; ref. 30) to secure tissues of the metastasis itself to use for prediction. This kind of question becomes all the more important in the setting of metastatic recurrence postadjuvant chemotherapy, as occurs with some frequency in bladder cancer, as such lesions are often chemotherapy-resistant and trials of second-line agents generally observe low response rates. Perhaps circulating tumor cells could be evaluated for the COXEN-derived GEMs (31), and this may be a solution that obviates the need for biopsy in patients in which such tumors can be captured and analyzed.

Conclusion

In summary, the COXEN algorithm shows promise as a means to use genomic information as a universal language to translate drug sensitivity biomarkers between cell line and tumor platforms. The findings reviewed herein constitute the first attempt at laying a foundation for future implementations that seem compelling and suggestive. Future studies ranging from model systems and preclinical studies to prospective clinical trials will determine if this concept, now in its infancy, will grow up into a strong adult in the fight against cancer.

Disclosure of Potential Conflicts of Interest

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References

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