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Lymphoblastic Cell Lines in Pharmacogenomics: How applicable are they in clinical outcomes?

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Opinion

LCLs are widely used in pharmacogenomic studies and the applicability of LCLs for various clinical phenotypes is emerging. Early studies have yielded promising results for LCLs as a proxy for genetic variants for treatment outcome for a number of cancers as well as toxicity in varying tissue types including taxane-induced neuropathy. Although LCLs have demonstrated utility in the elucidation of functional mechanisms for results of clinical genotype-drug phenotype studies, there are more relevant cell-based models developing.

Keywords

lymphoblastoid; pharmacogenetic; cell-based model; genotype-phenotype; cytotoxicity; apoptosis

Utility of lymphoblastoid cell lines as a Resource in Pharmacogenomic Discovery

The most relevant system to determine how an individual's genetic profile influences their likelihood of responding to a drug or developing toxicity would be humans; however performing appropriate pharmacogenomic trials requires a homogenous population of patients treated with the same dosage regimen with minimal confounding variables. Depending on the effect size, an adequately powered study could require thousands of patients. Furthermore, a replication trial is often necessary to increase confidence in the findings particularly if the genotypes are being considered for clinical implementation. In the field of oncology, this is difficult--if not impossible--because the standard of care tends to change as new therapies emerge and for most cancers, patients receive a multi-drug regimen. However, effective pharmacogenomic prediction in oncology would potentially have great utility because non-response and adverse events can be life threatening.

An approach for the identification of predictive markers that has emerged to overcome the difficulty of performing large clinical trials is the use of cell-based models. The most widely used model employs EBV-transformed LCLs containing publicly available genetic data

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from the International HapMap Project. Studies using LCLs from eleven different world populations to address inter-individual and inter-population comparisons have included a variety of cellular phenotypes that go beyond chemotherapeutic induced cytotoxicity and apoptosis including LDLR levels in response to simvastatin treatment [1], *Salmonella*-induced pyroptosis [2], radiation induced expression changes [3] and other phenotypes (reviewed in Wheeler and Dolan [4]). In addition to genetic (genotype and sequencing) data in the public domain, there are also baseline expression data using various array platforms [5–7], baseline microRNA data [8] and methylation data [9]. Genetic variants identified in genome wide association studies can be identified as potentially functional through their association with baseline mRNA expression, microRNA expression, or modified cytosines. The cells provide a cost-effective testing system in which environmental factors such as concomitant medications can be controlled [4].

Although there are a number of advantages of HapMap LCLs, the limitations include confounding variables such as cellular proliferation rate, EBV copy number, lack of expression of cytochrome P450 enzymes, and the fact that they represent one cell type have been a concern (reviewed previously [4, 10]). The fundamental concern of whether these LCLs recapitulate clinical outcomes is being addressed through various studies comparing LCL results with clinical outcomes.

Applicability of LCLs to clinically relevant phenotypes

Since lymphoblastoid cell lines arise from B-lymphocytes, one might assume they better represent blood-based toxicities, such as neutropenia, than toxicities in major organs such as nephrotoxicity or cardiotoxicity. Different expression patterns in tissues may account for differences in degree to which they are damaged by outside stimuli [11]. To no surprise, there is even greater skepticism that LCLs can identify relevant genetic variants predictive of tumor response, because of the many somatic changes and heterogeneity within tumors. However, targets discovered through pharmacogenomic studies in LCLs have been replicated in clinical trials, arguably the ultimate measure of utility [12, 13]. Top SNPs from LCLs associated with anti-leukemics were associated with clinical response in AML [14]. Those associated with platinum susceptibility were also associated with outcomes in head and neck cancer patients [15]; a top carboplatin SNP from LCLs was found to be significantly associated greater cellular sensitivity to carboplatin and when tested in ovarian cancer patients was associated with decreased progression free survival and poorer overall survival [16]. A recent study evaluating temozolomide response identified a SNP in the LCLs as associated with differential gene expression of MGMT ($p < 10^{-26}$) that was also associated with differential methylation in glioblastoma samples [17].

In addition, functional validation of clinical GWAS results in LCLs is also showing great promise. In cardiovascular pharmacogenomics, a haplotype associated in patients with poor simvastatin response was functionally evaluated in LCLs to discover a potential mechanism through alternative splicing in which the associated haplotype affected patient response [18]. In asthma pharmacogenomics, a genetic variant reproducibly associated in patient trials located in the promoter of glucocorticoid-induced transcript 1 (GLCCI1) was functionally

evaluated in LCLs. Utilizing LCLs, the variant was shown to influence expression of GLCCI1 and dexamethasone was shown to induce expression of GLCCI1 [19].

Understanding how well the overall genetic architecture of pharmacologic phenotypes can be captured by the cell-based models is of paramount importance. One approach recently developed that goes beyond evaluating top signals from LCLs in a patient population employed a permutation resampling analysis. The enrichment approach evaluates all SNPs with nominally significant associations to cytotoxicity in studies in cell-based models against those at least nominally significant in human clinical trials [20]. This was applied to GWAS results associated with paclitaxel-induced cytotoxicity in the LCL model and GWAS results of paclitaxel-induced neuropathy in patients. SNPs associated with patient paclitaxel-induced neuropathy were found to be enriched for SNPs associated with paclitaxel-induced cytotoxicity in HapMap LCLs and vice versa [21]. This significant enrichment confirms that LCLs are a useful model in the study of a subset of shared genes involved in patient toxicity. As more clinical GWAS results become available, the robustness of the LCLs for additional tissue types and toxicities can be evaluated.

Conclusion

The advantage to using cell-based models for pharmacogenomics discovery is the ability to perform experiments outside of humans in a well-controlled environment where *in vivo* confounders such as concomitant medications are eliminated. International HapMap LCLs also have the added advantage of a wealth of genetic, epigenetic, expression and pharmacologic data available. The full breadth of pharmacologic phenotypes that can be assessed in LCLs is not yet appreciated; however researchers have evaluated cell growth inhibition, apoptosis, gene expression modulation following drug treatment and other pharmacologic endpoints. Evidence comparing genetic predictors of patient outcomes with genetic predictors arising from LCL models has indicated robustness for the cell-based model. LCL identified genetic variants that correspond to treatment response in a number of diverse cancers include ovarian, head and neck, and leukemia and in toxicities such as peripheral neuropathy associated with paclitaxel. The use of enrichment studies between clinical GWAS signals in patient studies and LCL GWAS signals for a particular drug will help identify the utility of the cell-based model.

Future Perspective

As more pharmacogenomic clinical trials are completed and compared with LCL results for both overlap among top signals and enrichments among suggestive signals, we will have a better sense of where the LCLs are most useful in pharmacogenomic discovery. In general, clinical pharmacogenomics studies are hindered by the availability of well-phenotyped data in patients. The robustness of LCL predictors generated with the same agent will continue to be assessed clinically. To some degree, the applicability of LCLs in the discovery phase of genetic variants is primarily with oncology agents.

As studies linking genetic variants to mRNA, methylation, microRNA, and other functional units continue to grow, LCLs remain an attractive system to functionally evaluate genetic variants that are robustly associated with clinical phenotypes. Follow-up studies in LCLs can

link functional annotation to potential mechanism of associated signals for clinical studies. Furthermore, bringing in protein expression to the vast knowledge on these cells is an area of great interest. The application of micro-western arrays, which represent the latest generation of proteomic analysis methods for the large-scale analysis of protein abundance and modification will add great value to these cell-based models [22]. By using the micro-western arrays method one might uncover additional loci that may influence the translation or degradation rate of proteins that are key, high-level executors of cell decision making processes.

Another area of potential growth in pharmacogenomics is the use of well genotyped induced pluri-potent stem cells (iPS) that can then be converted to relevant tissues including neurons, hepatocytes, cardiomyocytes. These cell types have the potential for both discovery of genotype-phenotype relationships in tissues of toxicity and functional evaluation of potential mechanisms. A large repertoire of cell lines with known genetic background from different tissues of origin would be extremely beneficial to the field of pharmacogenomics.

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