Characterization of Cancer Gene Mutations in Human Cancer Cell lines for Correlation with Drug Activity

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This dissertation is submitted for the degree of Doctor of Philosophy

DECLARATION:

This dissertation is the result of my own work and includes work done in collaboration where specifically indicated in the text. The dissertation does not exceed the word limit set by the Biology Degree Committee.

Sequencing and mutational analysis of 24 cancer genes in the NCI-60:

The sequencing primers were designed to the 24 cancer genes by the bioinformaticians of the Cancer Genome Project. Optimization of the sequencing primers and preparation of the cell lines for sequencing was performed by the laboratory technicians of the Cancer Genome Project. The sequencing of the 24 genes in the NCI-60 cell lines was done through the Wellcome Trust Sanger Institute sequencing center.

I designed and built the custom *nci60* database to store all sequence variants identified. The perl script*, pass.fail* , was written by myself with help from Steffen Durinck, at the time, a fellow Ph.D. student at the European Bioinformatics Institute.

I identified 117 of the total 156 mutations using Mutation Surveyor v. 2.0 sequence analysis software and manual analysis. The Cancer Genome Project sequence analysis team members, consisting of approximately 24 individuals, identified the remaining 39 mutations using their in-house sequence analysis software (OncoCSA). Sudhir Varma performed the clustering analysis of the U133 expression data on the NCI-60.

Statistical analysis of mutations in cancer genes and drug activity in the NCI-60:

Mark Reimers, statistician at the National Cancer Institute, and Steffen Durinck, a bioinformatician at the National Cancer Institute, performed the statistical analysis of cancer gene mutation in the NCI-60 and activity of 7794 compounds. Paul Blower, a medicinal chemist at Ohio State University, performed the cheminformatic search for phenothiazine compounds tested in the National Cancer Institute, Developmental Therapeutics Program 60 cell line anti-cancer drug screen.

Statistical analysis of phenothiazines' activity in the NCI-60 and validation pharmacology experiments:

I performed additional statistical analyses on BRAF mutation in the NCI-60 and drug activity in the 7794 compounds and led the interpretation of the *in silico* results. I performed all of the subsequent pharmacology experiments, confirming the statistical association between BRAF mutation and phenothiazine activity and validating the predicted phenothiazines' antiproliferative activity in a larger set of cancer cell lines.

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"A word is dead when it is said, some say. I say it just begins to live that day".

---Emily Dickinson

ABSTRACT

The panel of 60 human cancer cell lines (the NCI-60) assembled by the National Cancer Institute for anticancer drug discovery is a widely used resource. The NCI-60 has been characterized pharmacologically and at the molecular level more extensively than any other set of cell lines. There has not, however, been a systematic sequence analysis of the NCI-60 for key genes causally implicated in oncogenesis. We report the sequence analysis of 24 known cancer genes in the NCI-60 and an assessment of four of the 24 genes for homozygous deletions. Using a pharmacogenomic approach, we have identified an association between mutation in BRAF and the anti-proliferative potential of phenothiazine compounds. Phenothiazine compounds have been used as anti-psychotics and as adjunct anti-emetics during cancer chemotherapy, and more recently reported to have anti-cancer properties. However, to date the phenothiazine anti-cancer mechanism of action has not been elucidated. We demonstrate that BRAF mutation (V600E) in melanoma is predictive of an increased sensitivity to phenothiazines. We also show that RAS mutant and RAS/BRAF wild type melanoma cell lines are approximately two-fold less sensitive to inhibition by phenothiazines than are BRAF mutant melanoma cell lines. This pattern of increased sensitivity to phenothiazines based on the presence of V600E BRAF mutation may be unique to melanomas; we do not observe it in a panel of colorectal cancers. The clinical implications for the use of phenothiazines in the treatment of melanoma, in light of the *in vitro* differential sensitivity between V600E BRAF mutant and RAS mutant melanomas are discussed.

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