Conclusions: At a non-toxic dose, Aza can reverse the hypermethylation in the human lung cancer cell lines and reexpresses tumor suppressor genes in mouse airway epithelium exposed to tobacco carcinogens. Regional administration to the airways enhances the therapeutic index of Aza by at least 75-fold. The potential of regional administration of Aza (including by aerosolization) for the treatment of advanced bronchial premalignancy deserves further investigation.

Pharmacogenetics

551 POSTER

Functional characteristics of human epidermal growth factor receptor (EGFR) polymorphisms

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Background: EGFR plays an important role in carcinogenesis and is an important target for cancer therapy. It is a trans-membrane protein with intrinsic tyrosine kinase activity which regulates signaling pathways that control cellular activities. Genetic alterations in *EGFR* have been implicated in a variety of human cancers, and have been shown to associate with variations in treatment outcomes mostly in Asians and Caucasians. We set out to systematically resequence the EGFR gene from African-American (AA) and Caucasian-American (CA) DNA samples and functionally characterize non-synonymous coding single nucleotide polymorphisms (cSNPs) observed.
Methods: All 28 *EGFR* exons, as well as splice junctions and portions of the 5′- and 3′- flanking regions were resequenced from 60 AA and 36 CA Coriell DNA samples. The non-synonymous *EGFR* constructs were transiently expressed in H727 lung cancer cells together with GFP to correct for transfection efficiency. A variety of functional parameters were measured.
Results: A total of 108 polymorphisms were observed, 3 of which were non-synonymous and changed encoded amino acids namely: Arg521Lys, His988Pro and Ser1162Asn. The His988Pro and Ser1162Asn were specific to the AA population and the Arg521Lys was observed in both populations. The 521Lys showed approximately 2.5 fold increase in levels of basal EGFR kinase enzyme activity and immunoreactive protein compared to either wild-type (WT), 988Pro or 1162Asn variant allozymes. The levels of enzyme activity and protein for 988Pro and 1162 Asn varaint allozymes however were similar to that of the WT. After 60 mins of stimulation in the presence of EGF ligand, phosphorylation (phosphor-EGFR) for the variant allozymes was reduced compared to the WT by 1.4-fold (988 Pro), 2-fold (1162Asn) and 2.5-fold (521Lys). Additionally, inhibitor characterization with erlotinib showed IC_{50} values ranging from 17.3 nM (521Lys), 41 nM (1162Asn), 46.3 nM (WT) to 65.8 nM (988 Pro). Cell proliferation in the presence of erlotinib was decreased for the 1162Asn and 521Lys variants compared to the WT and 988Pro variants.
Conclusions: The Lys521 polymorphism displayed increased basal kinase enzyme activity and protein levels but with higher inhibitor sensitivity and lower EGF stimulation compared to the 988Pro, 1162Asn and WT *EGFR* variants. These observations suggest that ethnic-specific pharmacogenetic variation in *EGFR* may contribute to efficacy and or toxicity during EGFR targeted therapy.

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CYP3A5*3 (rs776746) is associated with docetaxel-specific toxicities during adjuvant breast cancer chemotherapy

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Background: Systemic therapy is a commonly administered treatment modality in cancer care. Adverse drug reactions (ADR) during chemotherapy affect quality of life and may hinder delivery of adequate doses in a subpopulation of patients, thereby limiting the therapeutic benefit. The heritable nature of CYP gene polymorphisms influencing drug metabolism and clearance are well characterized. In this study we investigated germ-line polymorphisms in several of CYP genes including CYP3A5 (CYP3A5*3; G>A, rs776746); and drug efflux pump, MDR1 (C 3435 T, rs1045642) for their association with drug-induced toxicity phenotypes.
Methods: DNA samples from breast cancer clinical trial patients (n = 184) receiving adjuvant poly-chemotherapy regimen with docetaxel (Taxotere), doxorubicin (Adriamycin) and cyclophosphamide in Edmonton, (Alberta, Canada) were selected for this study. All subjects signed an informed consent and the study was approved by institutional research ethics board. Following stringent data filtering criteria, a total of 147 cases were considered for association analysis. Patients with drug induced toxicity scores of 0−2 (n = 57) served as a reference (controls); these were compared with $\geqslant$ grade 3 toxicity groups: overall toxicity (group I, n = 90), docetaxel-specific (group II, n = 36) and non-docetaxel related (group III, n = 54). Hypersensitivity, fatigue, myalgia and neurotoxicity were classified docetaxel specific. Genotyping was performed using the Pyrosequencing technology platform. Associations between genotype and phenotype were analysed using unconditional logistic regression with SNPStats software.
Results: We identified statistically significant associations only for CYP3A5 among several CYP gene polymorphisms tested. Group I (p-value 0.013; Odds Ratio (OR) 4.19 (CI: 1.17–15.03) and II (p-value 0.015; OR 5.14 (CI: 1.26–20.92)) results were consistent with the predictions that docetaxel-related toxicities are mediated predominantly by CYP3A5*3 (heterozygote and variant genotypes) conferring risk. The MDR1 polymorphism was not associated with either overall or docetaxel-specific toxicity; however, CC genotype (wild type allele conferring higher expression, i.e., clearance of docetaxel) showed marginal protection (OR 0.30, p-value 0.037) in non-docetaxel related toxicity in group III.
Conclusions: Pharmacogenetic screening helps stratify patients to (i) identify groups at risk for chemo-toxicity and (ii) customize therapies to improve treatment outcomes.

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The influence of CYP2D6 and CYP3A5 pharmacogenetics on pharmacokinetics of tamoxifen and its metabolites in Asian breast cancer patients

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Background: To investigate the impact of *CYP3A5*3*, *CYP2D6*5* and **10* polymorphisms on the plasma concentrations and metabolic ratios of tamoxifen (TAM) and its three metabolites, N-desmethyltamoxifen (NDM), 4-hydroxytamoxifen (4OHT) and endoxifen (END).
Methods: A total of 165 Asian breast cancer patients were genotyped for *CYP3A5*3* and *CYP2D6* polymorphisms. Plasma levels of TAM and its metabolites were determined at steady state using HPLC with fluorescence detection. Genotypic-phenotypic associations between genotypes were performed using Kruskal-Wallis test and Mann-Whitney U-test.
Results: The END level [median (range)] of patients with *CYP2D6*10/*10* [8.03 (1.74–34.68)] was significantly lower compared to patients with *CYP2D6*1/*1* [19.55 (4.18–39.47), p < 0.001] or *CYP2D6*1/*10* [19.74 (7.26–33.24), p < 0.001]. Similarly, the NDM level of *CYP2D6*10/*10* carriers [374.41 (84.77−802.98)] was significantly higher than *CYP2D6*1/*1* [174.59 (40.82–448.65), p = 0.001] or *CYP2D6*1/*10* [279.43 (115.41–502.13), p = 0.006] carriers. Higher plasma concentration ratio of END/(TAM+NDM)$\times 10^{-2}$ (TMR_{NDM}) was observed in patients with **1/*1* genotype in comparison to patients with **1/*10* and **10/*10* genotypes [**1/*1* vs **1/*10* vs **10/*10*: 5.03 (2.94–7.45) vs 3.91 (0.98–7.24) vs 1.50 (0.51–10.69), P < 0.001]. Likewise, the plasma concentration ratio of END/(TAM+4OHT)$\times 10^{-2}$ (TMR_{4OHT}) ratio was found to be lower in **10/*10* carriers [4.16 (1.14–17.05)] compared to **1/*1* [11.05 (6.09–14.73), P < 0.001] and **1/*10* [9.18 (2.39–18.70), P < 0.001] carriers. Although *CYP2D6*5* was not significantly associated with plasma levels of the analytes, patients carrying the **5* allele was associated with lower TMR_{NDM} [**1/*1* vs **1/*5*: 5.03 (2.94–7.45) vs 3.30 (2.61–4.56), P = 0.001] and TMR_{4OHT} [**1/*1* vs **1/*5*: 11.05 (6.09–14.73) vs 8.49 (5.26–11.77), P = 0.030]. No association between *CYP3A5*3* polymorphism and plasma concentrations of tamoxifen and its metabolites or the TMRs was observed.
Conclusion: *CYP2D6*5* and *10* polymorphisms were found to influence the plasma concentration and metabolic ratios of TAM and its metabolites in this exploratory study. The functional impact of polymorphisms present in genes encoding other enzymes involved in tamoxifen biochemical pathway should be investigated.