Pharmacogenomics of Weak Drug Effects

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Motivation

Microarray gene expression profiling is a powerful tool for the identification of drug effects in terms of gene regulation inasmuch as drug targets and affected pathways can be elucidated. Genomics is also suitable for comparative studies aimed at the identification of similar or overlapping drug effects. In fact, minor side effects may limit the utility of new drugs and should therefore be detected early during drug development. The power if microarray analyses is, however, limited by its intrinsic variability and by still relatively high costs that prohibit extensive screening needed to obtain statistically significant data for compounds with weak effects. We therefore set out to develop a procedure that would allow for screening weak effects in situations were a comparative compound with strong effects of the same type is available. As a test set we used phyto- (Curcumin, enterolactone, quercetin) and xenoestrogens (nonylphenol), compounds postulated to exert weak estrogenic effects, as well as the synthetic anti-estrogen, tamoxifen. The definition of estrogenic effects is important during drug development and at present, is carried out using cell proliferation assays.

Methods

We performed gene expression profiling of MCF7 cells treated for 72 hours with these compounds using Affymetrix HGU133plus2 arrays. Three independent biological replicates were analyzed. Overall effects of these compounds were very low and, when analyzed by standard statistical approaches (Significance Analysis of Microarray), no or very few genes passed the threshold. Then we performed the same analysis with 17-ï¿½estradiol (E2) as a positive control obtaining 334 statistically significantly more than twofold regulated genes (E2 genes). In order to assess the estrogenicity of phyto- and xenoestrogens, we performed Spearman correlations between the expression changes of the E2 genes induced by these compounds and those elicited by E2 and determined the significance of the correlations obtained. The ratio between E2 versus control over test compounds versus control was assessed and correlated to the extent of regulation by E2. This allowed for the identification of the estrogenicity of the compounds as a function of responsiveness to E2. E2 genes were divided into quintiles on the base of the extent of regulation by E2 and functional classes of the genes were analyzed.

Results

Correlations with varying significance(<0.0001 to 0.0118) were identified although most genes were regulated much more strongly by 17-ï¿½estradiol than by the other compounds in terms of fold change. Interestingly, the power of the phyto- and xenoestrogens was low for genes strongly regulated by 17-ï¿½Estradiol and similar to the latter for genes only weakly regulated by the classic estrogen. This allowed for the identification of functional classes of genes undergoing more or less prominent estrogenic effects when treated with phyto- and xenoestrogens. The procedure thus allows for the identification of weak drug effects that on their own do not reach statistical significance. The correlation r-value appears well suited for the definition of an objective threshold for a drug to be classified as having estrogenic effects.

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