Mechanistic Insights from Profiling Chemical-Mediated Transcription Factor Transactivation with the Integration of Cytochrome P450 Metabolism

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Profiling chemical effects on transcription factor activity is an important new approach methodology (NAM) that can help characterize the mechanisms by which chemicals may perturb biological systems. The Attagene cis-FACTORIAL™ assay uses a reporter system to detect activity of 46 transcription factors that provides a quantitative assessment of chemical effects. A new version of this assay, CYP-FACTORIAL™, includes addition of nine key cytochrome P450 (CYP450) enzymes to enable the evaluation of chemical effects on transcription factor activity with and without CYP-mediated phase 1 metabolism. This supports a better understanding of whether CYP-mediated oxidation results in an altered bioactivity profile.

The current study examined 24 expert-selected chemicals across four test concentrations in the cis-FACTORIAL and CYP-FACTORIAL assay formats. Results for this proof-of-concept study suggest that the transcription factors showing the greatest difference in response with CYP450 metabolism are those activating the estrogen receptor (ER), aryl hydrocarbon receptor (AhR), and oxidative stress response (nuclear factor erythroid 2–related factor 2 or NRF2) pathways. Comparisons of study chemical profiles to those of reference chemicals identified a highly conserved PAH toxicity signature involving activation of AhR, NRF2, and ER. Interestingly, a profile in which ER and AhR are activated but NRF2 is not activated correlated to non-toxic compounds, suggesting the possibility of using differences between signatures to predict toxic outcomes. Between profiling approaches and the integration of metabolism into a multiplexed in vitro assay system, this assay platform provides insight into chemically induced bioactivity and thus facilitates the development of mechanistically based, human-relevant NAMs. This project was funded with federal funds from NIEHS, NIH under Contract No. HHSN273201500010C..

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