"Phase-II metabolites as target biomarkers in doping analysis: generation of reference materials and methods optimization"

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Project Overview

In the fight against doping the laboratories are confronted with an increasing number of substances to screen on. Thus, a comprehensive screening for different classes of substances using dilute-and-inject methods in anti-doping screening is desirable. As lots of xenobiotics are excreted as conjugates a detection of the intact conjugates is performed by this approach. While chemical synthesis of phase-II metabolites works efficiently for compounds having only one potential conjugation site, several analogous compounds could not be chemically synthesized effectively, due to their more complex chemical structure. For the synthesis of the phase-II metabolites (glucuronides and sulfates) of these compounds a biotechnological production will be implemented. Fission yeast strains, that enable the biotechnological production of glucuronides and sulfates that cannot be synthesised efficiently via classical chemical synthesis will be generated and used to produce the relevant human conjugates. The produced reference material can be used for method set-up for direct detection. If laboratories still rely on hydrolysis of the conjugates, these reference compounds may serve as control for hydrolysis efficiency and quality assurance.

As proof of concept the use of the generated fission yeast strains will be demonstrated by generation of salbutamol-sulfate, salbutamol-glucuronide, fenoterol-sulfate and 4-hydroxy-DHEA-sulfate within the project.

Results and Conclusions:

A complete set of recombinant fission yeast strains each expressing one of the human sulfotransferases hSULT1A1, hSULT1A2, hSULT1A3, hSULT1B1, hSULT1C2, hSULT1C3a, hSULT1C3d, hSULT1C4, hSULT1E1, SULT2A1, hSULT2B1a, hSULT2B1b, SULT4A1, or hSULT6B1, respectively, was successfully generated. For each hSULT two strains were generated, one integrating the sequence in the leu1 gene of the cells, and an additional with a second expression unit in the pREP1-plasmid. Conjugation efficiency of the strains for the sulfonation of one test substrate (known from literature for the respective isoforms) for each SULT isoenzyme was successfully demonstrated. Ten out of twelve enzymes for which substrates are known were already shown to produce the respective sulfoconjugates. Analysis of the two remaining strains is in progress. These results prove that the intracellular production of the cofactor PAPS necessary for SULT activity in fission yeast is sufficiently high to support metabolite production by whole-cell biotransformation. We also developed a new and convenient SULT activity assay based on the sulfonation of a proluciferin compound and

established enzyme bag assays for SULTs. Taken together, we have developed the technology to systematically generate conjugates relevant for doping control by human SULTs.