DEVELOPMENT AND APPLICATION OF ACTIVITY-BASED HIF BIOASSAYS TO DETECT AND STUDY HIF HETERODIMERIZATION

Liesl Janssens and Christophe Stove

Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University

AIMS: Cells can sense oxygen levels and adapt cellular processes in low-oxygen environments. This hypoxic response is mainly mediated by hypoxia-inducible factors (HIFs). These heterodimeric transcription factors consist of a HIFα and HIFβ subunit. Under normoxic conditions, HIFα is actively degraded after hydroxylation by HIF prolyl- hydroxylases (PHDs), while under hypoxia the action of PHDs is impaired, causing accumulation of HIFα, resulting in increased formation of a functional heterodimeric HIF transcription factor. Transcriptional activity of HIF modulates the expression of a large panel of genes containing HIF-responsive elements (HREs), leading to the production of various proteins (e.g. erythropoietin). Pharmacological approaches to manipulate the HIF pathway by inhibiting PHD activity led to small-molecule HIF stabilizers as a new class of drugs to treat anemia. However, these drugs are also misused as performance enhancing substances. In this context, two cell-based activity-based bioassays were set up to serve both as a screening tool for doping control and to pharmacologically profile the HIF stabilizing effect. METHOD: The assays use the upstream mechanism of HIF activation (heterodimerization of HIF1α/2α with HIF1β) as a method for detection via fusing these proteins with parts of a split nanoluciferase to monitor protein-protein interaction. RESULTS: The newly developed HIF-bioassays are more sensitive than a traditional HRE- based gene-reporter assay (measuring downstream transcriptional read-out) and were successfully used to profile the activity of three HIF stabilizers, allowing to derive potency (EC50) and efficacy (Emax) values. CONCLUSION: The newly developed HIF-bioassays are promising platforms to detect and study HIF heterodimerization.