NON-GEL BASED PROTEOMICS TO STUDY STEROID RECEPTOR AGONSITS IN THE FATHEAD MINNOW

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Toxicoproteomics is an emerging field that is greatly enabled by non-gel based methods using LC MS/MS for biomarker discovery and characterization for endocrine disrupting chemicals. Using iTRAQ (isobaric tagging for relative and absolute quantitation), we quantified a diverse range of proteins in the liver and brain (telencephalon) of fathead minnows (FHMs) in response to androgenic (17β-trenbolone; Tb) and estrogenic (17α-ethinylestradiol; EE_2) pharmaceuticals respectively. In female FHMs exposed to 5 μg Tb/L for 48 hours, we detected altered hepatic proteins that were involved in metabolism (e.g., glyceraldehyde 3-phosphate dehydrogenase), general stress response (e.g., heat shock protein 60), and translational regulation (e.g., ribosomal proteins). Cell processes such as growth rate, cell division, differentiation, and glycolysis were candidate processes regulated via AR signaling. In male FHMs exposed to 5 ng EE_2/L for 48 hours, we identified altered proteins that were involved in cell structure (microtubule), metabolism (lactate dehydrogenase), and cell signaling (calmodulin). In addition, EE_2 affected proteins involved in oxidative stress, synaptic plasticity, and long-term potentiation. Exposure to both steroid receptor agonists affected cellular metabolism and stress responses. Gene expression analysis in the liver and brain of FHMs revealed that changes in mRNA levels may not always correlate to protein levels. Thus, the challenge of integrating proteomics and genomics data will need to consider temporal and regulatory relationships between genes and proteins for improved predictive power in risk assessment. We demonstrate the utility of non-gel based proteomics methods in aquatic toxicology and show that steroid receptor agonists rapidly alter the teleostean proteome.

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