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Review

Stressor-induced proteome alterations in zebrafish: A meta-analysis of response patterns

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ABSTRACT

Proteomics approaches are being increasingly applied in ecotoxicology on the premise that the identification of specific protein expression changes in response to a particular chemical would allow elucidation of the underlying molecular pathways leading to an adverse effect. This in turn is expected to promote the development of focused testing strategies for specific groups of toxicants. Although both gel-based and gel-free global characterization techniques provide limited proteome coverage, the conclusions regarding the cellular processes affected are still being drawn based on the few changes detected. To investigate how specific the detected responses are, we analyzed a set of studies that characterized proteome alterations induced by various physiological, chemical and biological stressors in zebrafish, a popular model organism. Our analysis highlights several proteins and protein groups, including heat shock and oxidative stress defense proteins, energy metabolism enzymes and cytoskeletal proteins, to be most frequently identified as responding to diverse stressors. In contrast, other potentially more specifically responding protein groups are detected much less frequently. Thus, zebrafish proteome responses to stress reported by different studies appear to depend mostly on the level of stress rather than on the specific stressor itself. This suggests that the most broadly used current proteomics technologies do not provide sufficient proteome coverage to allow in-depth investigation of specific mechanisms of toxicant action. We suggest that the results of any differential proteomics experiment performed with zebrafish should be interpreted keeping in mind the list of the most frequent responders that we have identified. Similar reservations should apply to any other species where proteome responses are analyzed by global proteomics methods. Careful consideration of the reliability and significance of observed changes is necessary in order not to over-interpret the experimental results and to prevent the proliferation of false positive linkages between the chemical and the cellular functions it perturbs. We further discuss the implications of the identified "top lists" of frequently responding proteins and protein families, and suggest further directions for proteomics research in ecotoxicology. Apart from improving the proteome coverage, further research should focus on defining the significance of the observed stress response patterns for organism phenotypes and on searching for common upstream regulators that can be targeted by specific assays.

Contents

Abbreviations: 2D-GE, two-dimensional gel electrophoresis; 2D-DiGE, two-dimensional difference gel electrophoresis; MS, mass spectrometry; mTOR, metazoan target of rapamycin; PTM, post-translational modification; SILAC, stable isotope labeling with amino acids in cell culture; SRM, selected reaction monitoring.

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1. Introduction

1.1. Toxicogenomics in environmental risk assessment

The major goal of ecotoxicology is to understand the effects of the environmental pollutants on living organisms in order to support the policies aimed at human and wildlife protection. Risk assessment requires characterization of both exposure and hazard components. Performing proper risk assessment is challenged by the steadily increasing number of commercially available chemicals that can potentially enter the environment (65,768,976 as of July 16th, 2014, of which 310,976 are inventoried/regulated substances, see http://www.cas.org/content/counter). Moreover, there is an alarming lack of toxicity data, even for single chemical compounds, not to mention mixtures that typically occur in the environment (Eggen et al., 2004; Eggen and Suter, 2007). The need to perform toxicity evaluations for a large number of compounds, imposed for instance by REACH regulation (Grindon and Combes, 2008), along with the lack of fully validated alternative testing methods for several complex toxicity outcomes of concern (Grindon et al., 2006; Lilienblum et al., 2008; Gundert-Remy et al., 2009), has been predicted to cause a major increase in the numbers of animals used for toxicity testing (Rovida and Hartung, 2009). Indeed, even though a recent report published by the European Chemicals Agency (ECHA) indicates that REACH registrants have increased their use of alternative methods, the number of toxicity tests performed on vertebrate animals has more than doubled during the same period (ECHA, 2014). Because the society strives to reduce the number of toxicity tests on animals, due to both ethical and economical reasons, the need for further development of alternative testing methods becomes obvious.

Toxicogenomics-based strategies aim to elucidate molecular mechanisms of action of different chemicals, with the ultimate goal of using this knowledge to develop focused alternative testing strategies for specific groups of toxicants. This approach is based on the assumption that physiological changes and toxic damage induced by chemicals are preceded by and reflected in molecular changes occurring in the exposed organisms. Thus, a detailed knowledge of the molecular processes involved is expected to promote the understanding of the mechanisms of toxicant action and identifying molecular biomarkers which can be used in prospective risk assessment as well as biomonitoring (Ankley et al., 2006; Boverhof and Zacharewski, 2006; Van Aggelen et al., 2010).

While classical approaches have focused on studying a few individual genes or metabolites at a time, "–omic" technologies have gained momentum over the last decade as a venue for characterizing the molecular changes and mechanisms defining specific states and metabolic capacities of an organism on a global scale (Garcia-Reyero and Perkins, 2011). The main "–omic" techniques are transcriptomics, proteomics and metabolomics, looking at changes in the composition and abundance of large numbers of mRNA transcripts, proteins, and small metabolites, respectively. The two former techniques provide direct information on changes in the expression of particular genes and will be discussed in more detail next.

1.2. Short overview of transcriptomics and proteomics technologies

Transcriptomic studies, classically performed using microarrays or, more recently, next-generation sequencing approaches, have served as a rich source of information on gene expression patterns characteristic of normal development, or evolving in response to diverse stimuli and stressors (Schirmer et al., 2010). However, compared to mRNA, proteins are perceived as being "closer to phenotype", reflecting the actual activity in the cells and providing more direct links to the regulated functions and the defense capacities of the organism under investigation. Thus, proteins are considered to be a more relevant gene expression level to look at (Rees et al., 2011; Diz et al., 2012). The mRNA abundance cannot be used as a direct prediction of protein expression levels, because correlation between the abundance of selected mRNA transcripts and corresponding protein products has been repeatedly shown to be rather poor (Washburn et al., 2003; Link et al., 2006; Wei et al., 2008; Groh et al., 2011a). Large scale analysis in mammalian cells suggested that differences in mRNA expression explain only 10–40% of the differences in protein levels (Schwanhaeusser et al., 2011), although later a refined analysis showed that the previous value was underestimated and could be 56–81% instead (Li et al., 2013). The discrepancies between the levels of mRNA and protein gene products are due to the fact that the rates of mRNA and protein synthesis and degradation are very diverse, with half-lives stretching over orders of magnitude and proteins generally being longer lived (Schwanhaeusser et al., 2011), and that expression levels can rapidly change in response to a stress. These uncertainties complicate the choice of matching analysis time points that would take into account the different rates of transcription, translation and degradation of mRNA and protein. In addition, diverse post-transcriptional and post-translational events involved in regulation of protein expression are not reflected in the transcriptomics datasets (Pradet-Balade et al., 2001). Therefore, it has been repeatedly argued that reliable information on gene expression changes influencing the changes in phenotype in response to a stress can only be obtained by performing dedicated proteomics analyses (Silvestre et al., 2012).

In a typical global differential proteomics experiment, control and case samples are compared. After treatment, proteins are extracted and analyzed either by gel-based or gel-free methods. Gel-based techniques include two-dimensional gel electrophoresis (2D-GE) (Gygi et al., 2000; Gorg et al., 2004) and its more powerful modification in terms of quantitation, two-dimensional difference gel electrophoresis (2D-DiGE) (Unlue et al., 1997; van