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Emerging systems biology approaches in nanotoxicology: Towards a mechanism-based understanding of nanomaterial hazard and risk

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ABSTRACT

Engineered nanomaterials are being developed for a variety of technological applications. However, the increasing use of nanomaterials in society has led to concerns about their potential adverse effects on human health and the environment. During the first decade of nanotoxicological research, the realization has emerged that effective risk assessment of the multitudes of new nanomaterials would benefit from a comprehensive understanding of their toxicological mechanisms, which is difficult to achieve with traditional, low-throughput, single end-point oriented approaches. Therefore, systems biology approaches are being progressively applied within the nano(eco)toxicological sciences. This novel paradigm implies that the study of biological systems should be integrative resulting in quantitative and predictive models of nanomaterial behaviour in a biological system. To this end, global 'omics' approaches with which to assess changes in genes, proteins, metabolites, etc. are deployed allowing for computational modelling of the biological effects of nanomaterials. Here, we highlight omics and systems biology studies in nanotoxicology, aiming towards the implementation of a systems nanotoxicology and mechanism-based risk assessment of nanomaterials.

1. Introduction

In the past decade, nanotoxicology has emerged as a specific domain within the toxicological sciences (Donaldson et al., 2004; Fadeel et al., 2013). In fact, we have witnessed an exponential rise in the number of papers on the subject, but as pointed out recently, nanotoxicology as a discipline is still struggling with the fundamental question: are there specific concerns associated with nanomaterials due to their particular or novel properties, that call for specific regulations to be applied in the case of nano-enabled products or technologies? (Krug, 2014). Furthermore, while considerable progress has been made, nanotoxicology still faces a number of challenges including the harmonization of nanoparticle dosimetry, the validation of in vitro assays for toxicity testing, and so on (Hussain et al., 2015). Indeed, while there are surely problems associated with many of the early papers in the field (Krug, 2014), one would be amiss to assume that all the papers published to date are of poor quality or that no lessons have been learned. Researchers are now fully cognisant of the importance of a thorough physicochemical characterization of the nanomaterials (Fadeel et al., 2015), and the role of the so-called biological "identity" of nanoparticles is also recognized (Nel et al., 2009; Monopoli et al., 2012). Nanotoxicologists have also understood that "not all nanomaterials are created equal" and that even slight differences in material properties could elicit a different biological response (Hussain et al., 2015). This, in turn, further emphasizes the need for a careful characterization of nanomaterials as well as standardized and validated procedures for toxicity testing, both in vitro and in vivo, to enable the comparison of results across different studies. However, to keep up with the rapid pace of development of new classes of nanomaterials of ever increasing sophistication, it is also clear that new approaches are needed in nanotoxicology; indeed, it may be argued that this is true for (regulatory) toxicology in general (Hartung, 2009). Understanding the potential health and environmental risks associated with exposure to chemicals and nanomaterials requires accurate and predictive risk assessment approaches. As pointed out in an excellent, recent perspective, developing such approaches requires a detailed mechanistic understanding of the ways in which substances perturb biological systems and lead to adverse outcomes (Sturla et al., 2014).

Systems biology approaches to human disease are grounded in the idea that diseases may perturb the normal network of a biological system through genetic perturbations and/or by pathological environmental cues (Hood et al., 2004). Systems biology has more recently been integrated with toxicology to give birth to *systems toxicology*, which

Abbreviations: AOP, adverse outcome pathway; CRP, C-reactive protein; 2D-DIGE, two-dimensional differential in-gel electrophoresis; ENM, engineered nanomaterial; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MIAME, Minimum Information About a Microarray Experiment; MIAPE, Minimum Information About a Proteomics Experiment; MS, mass spectrometry; NGS, next-generation sequencing; MWCNT, multi-walled carbon nanotube; NMR, nuclear magnetic resonance; NOTEL, no observed transcriptomic adverse effect level; OECD, Organisation for Economic Co-operation and Development; RNA-Seq, RNA sequencing.

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essentially aims at a holistic understanding of the mechanisms of interaction between substances and living systems at various levels of biological organization, in order to attempt computational modelling of complex toxicological pathways to ultimately support risk assessment (Sturla et al., 2014; Fadeel, 2015). To achieve such ambitious goals, systems toxicology must rely on accurate quantitative methods that enable the screening of a wide range of responses to a toxic insult. The ability to screen for multiple changes permits a much better understanding of toxicological pathways, in comparison to the traditional single end-point approach. To this end, so-called omics methods are being deployed; omics approaches are sometimes viewed as "high-throughput", but it can be argued that even though vast amounts of data are generated, this is not necessarily done in a high-throughput manner, as the data analysis can be demanding. In this context, *toxicogenomics* is a generic term commonly referring to molecular approaches to screen for alterations in gene expression and products of protein function in living systems subjected to toxicological challenge (Chen et al., 2012). The term comprises transcriptomics, proteomics, and other more recent approaches such as metabolomics and epigenomics, which are, in essence, related to different steps along the complex chain of events of gene expression and its consequences. Needless to say, computational tools and the ability to interpret complex pathways are of paramount importance. Indeed, as pointed out recently, systems biology should not be seen merely as the generation of lists of genes, proteins, or metabolites using omics platforms; the objective is to exploit these data and to develop quantitative, predictive models that describe the biological system and its response to individual perturbations (Fadeel, 2015). Notwithstanding, the application of omics in nanotoxicology is rapidly attaining maturity. Here, an overview is provided of omics techniques and their application in nanotoxicology, focusing mainly on work published in the last five years, and how this may contribute to a systems toxicology approach to support risk assessment. Published papers were selected that best served to illustrate the use of omics approaches to guide mechanism-based toxicological studies (see Table 1).

2. Omics and bioinformatics approaches

The suffix *-ome* as used in molecular biology refers to a *totality* of some sort; omics are thus used to assess globally all the genes, proteins, metabolites, etc. that are affected by a specific substance, or condition. Besides the ability to screen for multiple end-points in a single analytical run, omics techniques share the fact that they focus on changes at the molecular level. Technically, the methods applied differ according to their target, i.e., genes, transcripts, proteins, metabolites, and so on. We provide a brief description of omics and bioinformatics methods below, and how these different methods are being applied in nanotoxicology, and the reader is referred to more specialized reviews for further details. Additionally, it must be noted that systems toxicology is, by definition, a multi-level screening, which implies that the most informative research is likely that which integrates omics with more conventional end-points, to provide some measure of phenotypic anchoring of the data. Indeed, the key in systems biology (and, hence, in systems toxicology) is that phenotypic features of the system must be tied directly to the behaviour of the protein and gene regulatory networks (Ideker et al., 2001a). Moreover, systems biology, in essence, should capture *global* sets of biological information from as many hierarchical levels as possible (gene and protein regulatory networks, organs, individuals, populations, ecosystems) and integrate them (Ideker et al., 2001a).

2.1. Bioinformatics

The ultimate goal of systems biology is to produce predictive and preferably quantitative models of biological pathways, and computational tools therefore play a pivotal role. Bioinformatics provides crucial and ever-evolving tools for the analysis and interpretation of omics data. Specifically, bioinformatics can be deployed for the following three tasks in the toxicological sciences: (i) determination of which end-points (i.e., transcripts, proteins and others) are effectively de-regulated relatively to a control or calibrator plus the quantification of such changes; (ii) association of de-regulated end-points to specific biological pathways; and (iii) development of predictive models that can be used to support risk assessment (of chemicals, or nanomaterials) based on the understanding of complex networks of molecular interactions that are affected by exposure. The first task is rooted in the need to sort de-regulated end-points through adequate statistical processes that typically involve data normalization along with analysis of variance with false-positive discovery rates and other multiple test corrections of *p*-values. The second task serves to assist the complex data-mining process that follows the short-listing of end-points. In recent years, several bioinformatics tools have been developed for assessment of omics data, typically linked to public access databases with emphasis on genes, and proteins. As such, the first step of this task is to obtain annotations for datasets through database searches, followed by combining cluster analysis with functional annotation, based on, for instance, Gene Ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses. Clustering (of genes) aims for the identification of regulated biological processes through the evaluation of co-regulated genes. There are several software tools developed for the purpose for bioinformatics analysis of omics data, from R-based packages such as limma, minet and wgcna (with a range of algorithms for data normalization, statistics, clustering, etc), to more user-friendly web applications like BLAST (for sequence homology searches) and DAVID (for gene enrichment and other analyses) (McGinnis and Madden, 2004; Huang et al., 2009). Finally, building predictive models of toxicological pathways remains the major challenge. To this end, analytical methods such as the Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA) software offer a starting point to unravel dynamic biological pathways and networks (Calvano et al., 2005; Subramanian et al., 2005). In a vast majority of studies, a simple univariate strategy of testing the features (genes, transcripts, proteins, etc) one by one is used. However, this results merely in a list of differentially expressed or abundant molecules and does not necessarily provide information on their potential interactions. Also, in the context of identifying molecular biomarkers (see section below), this strategy has a number of limitations and more advanced feature selection methods using multivariate analysis are preferred, based on the assumption that subsets of interacting molecules should be identified at once (see Fortino et al., 2014 for some examples of such methods, implemented in R). It must be emphasized that there is a concern regarding the accuracy and reproducibility of omics data, which has led to proposals for a Minimum Information About a Microarray Experiment (MIAME) (Brazma et al., 2001) and a Minimum Information About a Proteomics Experiment (MIAPE) (Taylor et al., 2007) by bioinformatics experts as checklists of mandatory information to warrant validation. There are also efforts to amend these standards for specific applications, such as the MIAME/Toxicogenomics, or MIAME-Tox (Burgoon, 2007). In nanotoxicology, it is especially important that the omics experiments are performed using nanomaterials that are carefully characterized and that the models used are reliable and relevant; otherwise, one may run the risk of generating enormous amounts of useless data (Fadeel, 2015).

2.2. Transcriptomics

Transcriptomics aims essentially at quantifying changes in gene expression through detection of the number of mRNA copies. Unlike conventional qRT-PCR, transcriptomics technologies allow for the measurement of mRNA levels for thousands of genes simultaneously. Transcriptomics is likely the most common approach to survey both effects and mechanisms within the toxicological sciences and can be said to comprise of two distinct methods: oligonucleotide microarrays and next-generation sequencing (NGS), specifically, whole-transcriptome