

Nanotoxicogenomics: transcription profiling for the assessment of nanomaterials toxicity mechanisms

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Abstract

Recent studies show that certain nanomaterials (NMs) are toxic to living organisms, both in *in vitro* and *in vivo* studies. Main common mechanisms of toxicity, such as immunotoxicity, inflammation and increased production of oxygen reactive species which, in turn, provoke oxidative stress, have been already demonstrated for NMs. Nevertheless, specific mechanisms for different materials and key properties (i.e., solubility, size, shape) influencing toxicity remain to be elucidated. Toxicogenomics is a high throughput tool to investigate the molecular and cellular mechanisms of action of chemicals and other environmental stressors, including NMs, on biological systems, predicting toxicity before any functional damages, and allows classification of materials based on signatures of gene expression [1].

In this work, we studied the transcriptomic response in the liver of adult zebrafish (*Danio rerio*) exposed to 10 µg metal/L of CuO nanoparticles (NPs) of ~100 nm, maltose-coated Ag NPs of 20 nm and CdS quantum dots (QDs) of 3.5-4 nm, as well as to their ionic counterparts (CuCl₂, AgNO₃ or CdCl₂). After 3 and 21 days of exposure, the liver of 20 males was dissected out and a microarray study was performed using the Agilent technology Zebrafish (v3), 4x44k Gene Expression Microarray.

Copper exposures caused a weak effect on liver transcriptome compared to the response elicited by silver and cadmium compounds. CuO NPs differentially regulated 69 transcripts (LIMMA adjusted p<0.05 value) after 3 days of exposure while ionic copper significantly altered the expression of 30 transcripts after 21 days of exposure. Most of the GO terms (9 out of the 11) were shared in both treatments. General terms involving basic biological processes, such as “cellular process”, “single organism process”, “metabolic process”, “biological regulation” and “responses to stimulus” appeared enriched. Sequences related to “rhythmic process” were only regulated after 3 weeks of exposure to ionic copper. Exposure to silver for 3 days significantly regulated 243 different genes (Ag NPs) or 399 genes (ionic silver). After 21 days, the opposite trend was found: ionic silver regulated 265 transcripts and Ag NPs altered 990 different genes. At 3 days, ionic silver produced a strong disturbance of the energetic metabolism, inducing fatty acid catabolism, biosynthesis of unsaturated fatty acids and peroxisome proliferator activated receptor signaling pathway and inhibiting glycolysis, among others. All these alterations did not remain after 21 days. Exposure for 3 days to Ag NPs inhibited steroid biosynthesis and induced fatty acid catabolism and several amino acids catabolism. After 21 days, pentose phosphate pathway, ether lipid metabolism, most of the amino acids metabolism and many ribosomal proteins were up-regulated. CdS QDs significantly regulated 9 and 3638 genes after 3 and 21 days, respectively, while the ionic form regulated 37 and 11224 genes. Both cadmium forms up-regulated RNA degradation related biological processes after 21 days. In addition, ionic cadmium altered the DNA repair metabolism and down-regulated the carboxylic acid, alcohol and glycerol metabolic processes and amino acid and derivative metabolic processes, among others. QDs exposure inhibited actin cytoskeleton including focal adhesions and metabolism of carbohydrates and some amino acids, among others.

Overall, these results show distinct gene transcription signatures for the three metals, being the non-essential metals, silver and cadmium, those eliciting the strongest response in the zebrafish liver. Copper only altered general biological processes. In addition, transcription profiles distinguished metal forms (NP *versus* ionic form) and exposure times (3 *versus* 21 days), appearing as a useful tool to unveil nano-specific mechanisms of toxicity.

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References

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