Autophagy in Nanomaterial Toxicity

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Nanotechnology has rapidly promoted the development of a new generation of industrial and commercial products; however, it has also raised some concerns about human health and safety. To evaluate the toxicity of the great diversity of nanomaterials (NMs) in the traditional manner, a tremendous number of safety assessments and a very large number of animals would be required. For this reason, it is necessary to consider the use of alternative testing strategies or methods that reduce, refine, or replace (3Rs) the use of animals for assessing the toxicity of NMs. Autophagy is considered an early indicator of NM interactions with cells and has been recently recognized as an important form of cell death in nanoparticle-induced toxicity. Impairment of autophagy is related to the accelerated pathogenesis of diseases. By using mechanism-based high-throughput screening in vitro, we can predict the NMs that may lead to the generation of disease outcomes in vivo. Thus, a tiered testing strategy is suggested that includes a set of standardized assays in relevant human cell lines followed by critical validation studies carried out in animals or whole organism models such as C. elegans (Caenorhabditis elegans), zebrafish (Danio rerio), and Drosophila (Drosophila melanogaster)for improved screening of NM safety. A thorough understanding of the mechanisms by which NMs perturb biological systems, including autophagy induction, is critical for a more comprehensive elucidation of nanotoxicity. A more profound understanding of toxicity mechanisms will also facilitate the development of prevention and intervention policies against adverse outcomes induced by NMs. The development of a tiered testing strategy for NM hazard assessment not only promotes a more widespread adoption of non-rodent or 3R principles but also makes nanotoxicology testing more ethical, relevant, and cost- and time-efficient.

nanomaterials autophagy alternative testing strategy high throughput screening

1. Nanomaterials (NMs)

Nanomaterials (NMs) are defined as having at least one dimension that is 1â100 nm in diameter [1] and unique properties; for example, they can change reactivity, optical characteristics, or conductivity, thereby enabling novel applications. Furthermore, particle properties can be modified to promote different applications, resulting in consumer benefits, particularly in medical and industrial applications [2]. In recent years, nanotechnology has rapidly been promoted in the development of a new generation of industrial and commercial products. It has been estimated that the nanoproduct demands in medicine and pharmaceuticals, and especially the cosmetics industry, are expected to rise by over 17% each year and at a much higher rate in the food industry [3][4]. However, the application of nanotechnology has also raised some concerns about human health and safety. In some cases, nanomaterials present unexpected risks to both humans and the environment. Regulatory authorities in the European Union, United States, and Asian countries carefully observe developments in nanotechnology, trying to find a balance between consumer safety and the interests of the industry [5]. In addition, several international planning activities have been proposed or performed with the expectation that significant advances will be made in understanding the potential hazards triggered by nanomaterial exposure in both occupational and consumer environments [2].

2. Potential Hazards

Assessments of the potential hazards associated with nanotechnology have been emerging, but substantial challenges remain because all of the different nanoparticle (NP) types cannot be effectively evaluated for safety and environmental effects in a timely manner [2]. Identification of the physicochemical properties of nanomaterials that confer toxicity is a core component of toxicity studies. To evaluate the toxicity of the great diversity of NMs, a tremendous number of safety assessments would need to be conducted. It was estimated that, in 2009, a complete toxicity evaluation of all the nanomaterial on the market using traditional animal approaches would cost more than 1 billion US dollars, take at least 50 years, and require a very large number of animals [6][7]. Whether animals can be used to predict human response to toxicant exposure is still under debate, attributing to data gap between human and animal studies. Thus, there is a need for developing and using human-cell-based methods that generate human-relevant mechanistic data that are not necessarily obtainable from traditional animal studies conducted by vertebrate animals [8][9][10]. Furthermore, there are government regulations that have resulted in an enhanced need for alternative methods, such as the E.U. Cosmetics Directive that prohibits the testing of cosmetics products on animals in the European Union (EU Regulation 1223/2009). For all these reasons, it is necessary to consider the use of alternative testing strategies or methods that reduce, refine, or replace (3Rs) the use of animals for assessing the toxicity of nanomaterials [10].

Alternative testing strategies are commonly used to assess the safety of chemicals, and many of these strategies have been evaluated for their applicability to the testing of nanomaterials. A single alternative testing method may contribute to basic mechanistic or toxicity knowledge but may not be sufficient for use in hazard assessment. However, incorporating multiple alternative testing methods into alternative testing strategies will provide an understanding of the behavior and toxicity of nanomaterials in humans and the environment [10][11]. In vitro testing was proposed as the principal approach with the support of in vivo assays to fill knowledge gaps, including tests conducted in non-mammalian species such as C. elegans, Drosophila, and zebrafish, or genetically engineered animal models. These tools are being used to identify responses in cells exposed to chemicals expected to result in toxic effects [9][12]. Well-designed alternative testing strategies will not only allow for the prioritization of nanomaterials for further testing but can also assist in the prediction of risk to human beings and the environment.

3. Autophagy

Autophagy is a catabolic mechanism that is evolutionarily conserved from yeast to mammals. The autophagy pathway first described by Christian De Duve in 1963 [13] is a ubiquitous process that involves the degradation of cytoplasmic components and cytoplasm organelles, that degrade through the lysosomal pathway, and is distinct from other degradative pathways, such as proteasomal degradation [14]. When energy is limiting (ATP shortage), AMP kinase (AMPK) is activated, which can drive autophagy. Similarly, deprivation of growth factors or amino acids leads to the inhibition of TORC1, which is a repressor of conventional autophagy [15]. The inability to regulate autophagy is associated with aging, neurodegeneration, and a variety of diseases, including cancer, type 2 diabetes, and atherosclerosis [16]. Autophagy was recently recognized as an important form of cell death in various types of nanoparticle-induced toxicity, but the details of the underlying mechanisms are still unclear. A thorough understanding of the cellular and molecular mechanisms of nanoparticle-triggered toxicity is critical for a more comprehensive elucidation of nanotoxicity [17]. Our recent work provides the first demonstration that autophagy activated by silver nanoparticles (AgNPs) in normal cells fails to trigger lysosomal degradation pathway and led to a toxicity phenomenon called defective autophagic flux or autophagy dysfunction, which is relevant to the accelerated cellular pathogenesis of diseases [18][19][20]. The toxic effects induced by AgNPs and some of the metal oxide NPs, such as ZnONPs, have been shown, either in vitro or in vivo, to be quite similar in terms of cytotoxicity, genotoxicity,

hematotoxicity, immunotoxicity, hepatotoxicity, and embryotoxicity [19][21]. A more profound understanding of these toxicity mechanisms will facilitate the development of prevention and intervention policies against adverse outcomes induced by metal and metal oxide nanomaterials. Knowledge derived from the cellular and molecular processes underlying nanomaterial-induced toxic effects may also facilitate the establishment of the scientific foundations of nanomaterial risk assessment. Therefore, an overview of current findings regarding the mediation of autophagy triggered by NPs both in vitro and in vivo will shed light on the pivotal role of autophagy in nanomaterial toxicity and the useful implementation of autophagy in safety assessments conducted through alternative testing strategies.

References

1. European Commission. Commission Recommendation of 18 October 2011 on the definition of nanomaterial. J. Off. Eur. Union 2011, 275, 38â40.
2. Warheit, D.B. Hazard and risk assessment strategies for nanoparticle exposures: How far have we come in the past 10 years? F1000Research 2018, 7, 376.
3. Thomas Peter; Uwe Vohrer; Heike Mertsching; In vitro toxicity assessment of carbon nanomaterial dispersions. *Toxicology Letters* **2007**, *172*, S106-S107, 10.1016/j.toxlet.2007.05.287.
4. Aditi Jain; Shivendu Ranjan; Nandita Dasgupta; Chidambaram Ramalingam; Nanomaterials in food and agriculture: An overview on their safety concerns and regulatory issues. *Critical Reviews in Food Science and Nutrition* **2017**, *58*, 297-317, 10.1080/10408398.2016.1160363.
5. Matthias G. Wacker; Ana Proykova; Gustavo Mendes Lima Santos; Dealing with nanosafety around the globeâRegulation vs. innovation. *International Journal of Pharmaceutics* **2016**, *509*, 95-106, 10.1016/j.ijpharm.2016.05.015.
6. Jae-Young Choi; Gurumurthy Ramachandran; Milind Kandlikar; The Impact of Toxicity Testing Costs on Nanomaterial Regulation. *Environmental Science & Technology* **2009**, *43*, 3030-3034, 10.1021/es802388s.
7. Helinor Johnston; Rachel Verdon; Suzanne Gillies; David M. Brown; Teresa Fernandes; Theodore B. Henry; Adriano G. Rossi; Lang Tran; Carl Tucker; Charles Tyler; et al. Adoption of in vitro systems and zebrafish embryos as alternative models for reducing rodent use in assessments of immunological and oxidative stress responses to nanomaterials. *Critical Reviews in Toxicology* **2017**, *48*, 252-271, 10.1080/10408444.2017.1404965.
8. Robyn L Prueitt; B D Beck; Commentary on ääToxicity Testing in the 21st Century: A vision and a Strategyää. *Human & Experimental Toxicology* **2010**, *29*, 7-9, 10.1177/0960327109354661.
9. National Research Council. Toxicity Testing in the 21st Century: A Vision and a Strategy; National Academies Press: Washington, DC, USA, 2007; ISBN 978-030-910-992-5.
10. J. A. Shatkin; Kimberly J. Ong; Christian Beaudrie; Amy J. Clippinger; Christine Ogilvie Hendren; Myriam Hill; Patricia Holden; Alan J. Kennedy; Baram Kim; Margaret MacDonell; et al. Advancing Risk Analysis for Nanoscale Materials: Report from an International Workshop on the Role of Alternative Testing Strategies for Advancement. *Risk Analysis* **2016**, *36*, 1520-1537, 10.1111/risa.12683.
11. M DusinskÃ¡; S Boland; Margaret Saunders; L. Juillerat-Jeanneret; L. Tran; G. Pojana; A. Marcomini; K. Volkovova; J. Tulinska; Lisbeth E. Knudsen; et al. Towards an alternative testing strategy for nanomaterials used in nanomedicine: Lessons from NanoTEST. *Nanotoxicology* **2015**, *9*, 118-132, 10.3109/17435390.2014.991431.

12. Francis S. Collins; George M. Gray; John R. Bucher; Transforming Environmental Health Protection. *Science* **2008**, *319*, 906-907, 10.1126/science.1154619.
13. Christian De Duve; The Lysosome. *Scientific American* **1963**, *208*, 64-73, 10.1038/scientificamerican0563-64.
14. N. Mizushima; Autophagy in Protein and Organelle Turnover. *Cold Spring Harbor Symposia on Quantitative Biology* **2011**, *76*, 397-402, 10.1101/sqb.2011.76.011023.
15. Douglas R. Green; Beth Levine; To be or not to be? How selective autophagy and cell death govern cell fate. *Cell* **2014**, *157*, 65-75, 10.1016/j.cell.2014.02.049.
16. Donna Denton; Sharad Kumar; Autophagy-dependent cell death. *Cell Death & Differentiation* **2018**, *26*, 605-616, 10.1038/s41418-018-0252-y.
17. Yubin Li; Dianwen Ju; The Role of Autophagy in Nanoparticles-Induced Toxicity and Its Related Cellular and Molecular Mechanisms. *Results and Problems in Cell Differentiation* **2018**, , 71-84, 10.1007/978-3-319-72041-8_5.
18. Yu-Hsuan Lee; Fong-Yu Cheng; Hui-Wen Chiu; Jui-Chen Tsai; Chun-Yong Fang; Chun-Wan Chen; Ying-Jan Wang; Cytotoxicity, oxidative stress, apoptosis and the autophagic effects of silver nanoparticles in mouse embryonic fibroblasts. *Biomaterials* **2014**, *35*, 4706-4715, 10.1016/j.biomaterials.2014.02.021.
19. Bin-Hsu Mao; Jui-Chen Tsai; Chun-Wan Chen; Shian-Jang Yan; Ying-Jan Wang; Mechanisms of silver nanoparticle-induced toxicity and important role of autophagy. *Nanotoxicology* **2016**, *10*, 1021-1040, 10.1080/17435390.2016.1189614.
20. Yu-Hsuan Lee; Chun-Yong Fang; Hui-Wen Chiu; Fong-Yu Cheng; Jui-Chen Tsai; Chun-Wan Chen; Ying-Jan Wang; Endoplasmic Reticulum Stress-Triggered Autophagy and Lysosomal Dysfunction Contribute to the Cytotoxicity of Amine-Modified Silver Nanoparticles in NIH 3T3 Cells. *Journal of Biomedical Nanotechnology* **2017**, *13*, 778-794, 10.1166/jbn.2017.2395.
21. Lingling Ding; Zhidong Liu; Mike Okweesi Aggrey; Chunhua Li; Jing Chen; Ling Tong; Nanotoxicity: the toxicity research progress of metal and metal-containing nanoparticles.. *Mini-Reviews in Medicinal Chemistry* **2015**, *15*, 529-542, 10.2174/1389557515071504241043334.