**Turning a frown upside down: Exploiting nanoparticle toxicity for anticancer therapy.**

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The use of nanosized materials in cancer therapy is being extensively studied in trying to optimize multiple factors such as targeting efficacy, controlled drug release or externally triggered hyperthermia1. Recently, several groups have explored a novel nanomaterial-based therapy, exploiting the intrinsic toxicity of several types of nanomaterials. Various studies have found that many different types of nanomaterials can induce cellular autophagy and/or cell death by ion leaching2, 3. Interestingly, the extent of these effects appears to be highly dependent on the nature of the cells, as cancerous cell types were found to be far more susceptible than non-cancerous cells4. Both *in vitro* and *in vivo* data have demonstrated the potential of these methodologies.

Although the field of nanotoxicology remains under full development, several common mechanisms have been found and in general, there is a reasonable control over the extent of nanomaterial-related toxicity. Therefore, researchers believe that the intrinsic toxicity of nanomaterials could be exploited as a novel anticancer therapy5. Currently, two strategies have attracted special interest by multiple research groups: 1) the induction of autophagy by a wide variety of nanomaterials and 2) the leaching of metal ions by metallic or metaloxide particles (**Figure 1**).

In cancer research, the controlled induction of autophagy, an essential catabolic process by which cells break down their own components ultimately resulting in either cell survival or death6, is being actively studied7. A wide variety of different types of nanomaterials have been found to induce autophagy2, as exemplified in **Table 1**. A full overview of all nanomaterials linked to autophagy can be found in a recent report by Stern et al.8. Interestingly, several recent studies have compared the autophagy-inducing effects of nanomaterials in both cancer and non-cancer cell lines, observing a clear selectivity of the particles towards cancer cells4, 9. Both iron oxide9 and iron core/gold shell nanoparticles4 have been found to selectively induce autophagy and eventually cell death in cancer cell lines. The combined use of nanomaterials and chemotherapeutics furthermore enhanced their anticancer efficacy at significantly lower concentrations, minimizing any undesired side-effects. Yokoyama *et al*.10 used an antibody targeted against the epidermal growth factor receptor (EGFR) as therapeutic agent and observed that, when conjugated to plasmonic magnetic nanoparticles, this resulted in a clear synergistic effect and enhanced cancer cell killing.

One key aspect that needs to be resolved, is the possibility of tightly controlled induction of autophagy by the nanomaterials. Important possibilities in this aspect are the size of the particles and their level of cellular uptake. Furthermore, although many different nanomaterials have been linked to autophagy, we believe that it is essential to try and define which materials have the highest potency for autophagy induction and by which mechanism they induce it, in order to try and find the optimal formulation for cancer therapy. A nice breakthrough in the controlled induction of autophagy was recently reported by Zhang *et al*.3 who employed lanthanide oxide and upconversion nanoparticles (UCP) as autophagy-inducing nanomaterials. They then coated them with a series of short synthetic peptides which showed different potencies in reducing autophagy induction, rendering it possible to carefully tune the extent of autophagy by varying the coating peptides on the particles. Furthermore, using *in vivo* experiments, the authors did not observe any toxic effects of the particles on healthy tissues.

The underlying mechanisms causing nanomaterial-induced autophagy are still largely unknown, but several findings have pointed to the involvement of the classical Akt/mTOR pathway11, 12. We hypothesize that the persistence of endosomally located nanomaterials that are not or only slowly degradable (metallic particles or synthetic molecules (e.g. cationic lipids)13) can reduce the degradative capacity of the cells. Similarly, cellular degradation may also be impeded by materials that have an alkalizing effect and augment lysosomal pH14. The cellular degradative capacity is controlled by the CLEAR gene network (Coordinated Lysosomal Expression and Regulation), which is regulated by transcription factor EB (TFEB)15. Nanomaterial-related effects on the cells’ degradative capacity can either directly induce autophagy by nuclear translocation of TFEB or by reducing autophagy flux, resulting in an accumulation of autophagosomes (**Figure 1B**).

The potential of controlled autophagy induction by nanomaterials could have big impacts on many aspects of human health as autophagy has become increasingly associated with various pathologies16. As such, induction of autophagy could be used to help in the treatment of immune-related diseases such as Crohn’s disease, neurodegenerative diseases such as Huntington or familial Parkinson’s by clearance of misfolded proteins and protect cardiac cells from ischemia/reperfusion injury upon heart failure16.

Although the results are very promising, several challenges still need to be overcome. For a good antitumoral effect, we believe it to be essential that the materials are specifically delivered to the site of interest and homogeneously distributed in the tumor mass. Additionally, autophagy is a complex process and is linked to many other signal transduction pathways, where it can result in either cytoprotection or apoptosis. Therefore, we believe that more information is required on how the nanomaterials induce autophagy and to try and find ways to ensure that the desired pro- or anti-survival effects are obtained.

Most metal ions are known to be quite toxic and the contribution of these ions to the toxicity of metal and metaloxide nanoparticles has long been an issue of debate. Recently, Xiu *et al*.17 was able to answer this question, by demonstrating that Ag+ is the molecular toxicant of silver nanoparticles. Upon exposing bacterial strains under anaerobic conditions, thus precluding oxidation and Ag+ release, particles were non-toxic. In contrast, the particles were found to be toxic in a normal atmosphere where they release Ag+.

Although the slow dissolution of metal or metaloxide nanomaterials may render the particle suspensions toxic upon long-term storage, the intracellular stability of the particles is equally crucial. The release of metal ions occurs much more rapidly at lower pH levels, such as in cellular lysosomes. Using lysosomal buffer systems, iron oxide and quantum dot particles showed a clear pH-dependent release of metal ions18, 19. It has been found that coatings can influence the nanoparticle degradation kinetics18. For instance, silica/polymer double coated quantum dots were found to be much more stable at low pH levels as compared to single coated quantum dots20. Additionally, the nature of the particle core itself plays an important role. For example, ZnO nanoparticles typically dissolve rapidly within a cellular endosome21, but doping the particles with iron reduces the degree of degradation and allows to tune the level of toxicity22. While several studies have shown that administration of free ions resulted in a lower toxicity than whole nanoparticles, this is likely because endosomal uptake of nanoparticles results in a gradual, but long-term release of metal ions, reaching very high local ion concentrations that are not obtained by administration of free ions.

The effect of the ions on cell viability depends on the nature of the metal ion, where Fe2+ is tolerated much better than Cd2+. Interestingly, free metal ions such as Zn2+ or Ag+ have shown to have drastic effects on cell proliferation23. Both silver and ZnO particles were found to selectively inhibit the proliferation of rapidly dividing cancer cells in comparison to normal cells24, 25. Several recent studies have shown a lot of potential for degrading ZnO or silver nanoparticles as novel tools in cancer therapy. Sasidharan *et al*.26 showed that ZnO particles degraded much faster in the low local pH environment typically associated with tumors, hereby selectively killing cancer cells. Muhammad *et al*.27 used ZnO nanolids to seal the pores in doxorubicin-loaded mesoporous silica particles. Upon endosomal dissolution of ZnO, this resulted in a synergistic effect of both Zn2+ ions and free doxorubicin on cancer cells. Interestingly, Liu *et al*.28 employed TAT-peptide derivatized silver NPs and found that these particles were highly efficient against multidrug resistant cancer cells, which the authors postulated to be a size-dependent effect as the particles may have been too large to be excreted. Furthermore, in mice bearing malignant melanoma the particles efficiently inhibited tumor growth (**Figure 2**), without causing any observable effects elsewhere.

In both cases, the nano-size of the materials is essential. Preferably, the particles should end up in the degradative endosomal micro-environment, should be highly taken up by cells and for *in vivo* applications, must be efficiently delivered to the desired target site. Therefore, the particles must be in the nanometer-range. Furthermore, compared to chemicals, these nanomaterials can further be used as carriers for synergistic effects of both the particles and common therapeutics. Additionally, the particles can easily be coupled to various agents for targeted delivery.

In conclusion, these data demonstrate the huge potential that nanomaterials possess to be further developed as anticancer agents. The intrinsic toxicity of a wide variety of materials and the selectivity thereof towards cancer cells makes this a facile and universal methodology. Although several *in vitro* studies have already demonstrated the potential of this technology, more data is needed to fully unravel the mechanisms involved. Additionally, the use of more complex culture models such as cell cocultures or 3D tissues together with animal studies should be used to further verify the selectivity of this method. Additionally, more validated methodologies are needed that allow to study the stability of the particles in a complex biological matrix. Finally, optimization of both particle and surface chemistries should occur to enable fine tuning of the dissolution kinetics for minimal danger towards healthy cells but maximal efficacy against cancer cells. Taken all these factors into account and the recent surge in interesting findings, we believe that this technology will rapidly grow to become one of the main research foci in nanomedicine.

**Table 1. An overview of several nanomaterials associated with autophagy and their main mechanisms described thus far.**

|  |  |  |
| --- | --- | --- |
| NP | Important features | Refs |
| Gold NPs | Reported to have an alkalizing effect, impeding autophagy flux; combined with oxidative stress; selectively kills cancer cells | 4, 14, 29 |
| Quantum dots | Size-dependent activation of autophagy by direct induction via the Akt/mTOR pathway; cellular adaptation to oxidative stress by autophagy induction | 2, 30, 31, 32 |
| Polymer-based NPs | Induction of oxidative stress results in upregulation of autophagy via mTOR pathway | 11, 33 |
| Iron oxide NPs | Induction of oxidative stress and associated toxicity; upregulation of autophagy as secondary defense mechanism | 34, 35 |
| Carbon-based materials | Induction of oxidative stress; activation of mTOR pathway | 12, 36-38 |

**Figures.**

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**Figure 1.** **Schematic illustration of nanomaterial-induced toxicity by A,B) autophagy and C) metal particle degradation.** A) Overview of how nanomaterials can result in autophagy, by either direct induction of autophagy (1) or by inhibiting autophagy flux (2), the process by which autophagosomes fuse with lysosomes to be degraded as autophagolysosomes. B) Illustration of the mechanisms underlying autophagy induction, direct induction is due to (1) lower endosomal activity caused by the persistence of nondegradable materials, resulting in activation of TFEB and the Akt/mTOR/p70S6K pathway. Inhibition of autophagy flux (2) is due to lowered lysosomal activity, impeding the fusion of autophagosomes with lysosomes and hereby lowering autophagy flux. C) Endo- or lysosomal confinement of metal-based nanomaterials can result in the degradation of the particles, releasing free metal ions such as Cd2+, Zn+, Ag+. The gradual, long-term release of these ions and the high local concentrations they reach can then result in cell death.

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**Figure 2.** **The *in vivo* anticancer activity of nanosilver**. Photomicrographs of representative tumors excised from animals treated with the conditions indicated (saline; silver NP (0.5 nmol/kg; 1.0 nmol/kg); TAT-conjugated silver NPs (0.5 nmol/kg; 1.0 nmol/kg) and doxorubicin (2.6 µmol/kg; 4.3 µmol/kg)) showing their individual sizes. This image has been reproduced with permission from Liu *et al*.28 © Elsevier.

**Acknowledgements.**

SJS is a post-doctoral fellow from FWO-Vlaanderen. The FWO-Vlaanderen is acknowledged for their financial support (Krediet aan Navorsers). This work is funded by the Centre for Nano- and Biophotonics.

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