Commentary

Playing with nanoparticle shapes and laser powers to decide which route to take during photothermal therapy: apoptosis or necrosis?

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Due to the high stability and biocompatibility gold nanoparticles (AuNP) are extensively exploited for biomedical purposes. The easy synthesis methods and reproducibility and the possibility to tailor the surface with functional molecules make AuNP excellent candidate for diagnostics and therapeutics (1-3). Moreover, for those biomedical applications that require a considerably deeper penetration of near-infrared (NIR) light, in which both blood and soft tissues are highly penetrable, a different type of gold nanostructure is required, presenting plasmonic properties (4). Surface plasmon resonance is a phenomenon in which free electrons in the nanostructures collectively oscillate and scatter or absorb the incident electromagnetic wave. The rapid relaxation of the excited electrons produces strong localized heat which can be exploited for several applications spanning from photothermal therapy to optoacoustic imaging (5), drug delivery (6) and biosensing (7). Among plasmonic materials (i.e., materials able to convert light into heat) AuNP shaped as nanoshells, nanorods and nanocages may act as superb nanoheaters, developing amount of heat suitable to destroy living cells. Recently, de la Fuente and co-workers developed a high-yielding synthetic method for producing anisotropic gold nanoprisms (NPRs) (8), shown highly efficient nanoheaters both *in vitro* (8) and *in vivo* (9), expanding the class of gold plasmonic materials.

Despite the recent advances in designing novel structures, shapes, and surface coating to enhance photothermal effect, the responses elicited in the cells upon NIR irradiation remain largely unknown, which hamper the translation of photothermal therapy mediated by nanoparticles into clinics. Several works report on the necrosis as the most common cellular response. However, most experimental evidences supporting this conclusion consist on cell viability assays based on testing membrane integrity (AnnexinV-calcein/Propidium Iodide staining, or lactate dehydrogenase assay) (10) and monitoring very late stage of necrosis, when membrane integrity is irreversibly compromised. But how about monitoring the initial stage of cell death? How to control the pathway elicited in a cell by a plasmonic material and drive its route to death? The possibility to modulate the intrinsic properties of plasmonic nanomaterials by changing size, shape and composition enable a first control of the amount of heat generated upon irradiation. However, the cascade of downstream events induced in the treated cells need to deeply characterized before designing any therapeutic device.

Necrosis, once thought as simply a passive, unorganized way to die, has emerged as an alternate form of programmed cell death whose activation might have important biological consequences, including the induction of an inflammatory response. Although initially thought to constitute mutually exclusive cellular states, recent findings reveal cellular contexts that require a balanced interplay between apoptosis and necrosis (11,12). Thus what happen in a cell following nanoparticle mediated NIR irradiation? Addressing this issue by characterization of early and late stages of cell death pathways may open new possibilities in drug discovery and photothermal therapy. A first study performed on PC cells treated with multiwalled carbon nanotubes the mitochondrial membrane permeabilization with consequent apoptosis has been shown the primary mode of death induction following laser irradiation (13). In line with these finding, a recent

interesting article published on *ACS Nano* from de la Fuente group consider the opportunity to evaluate the molecular mechanisms induced in cells by NPRs upon irradiation (14). Aware that "*the cellular response of cells containing plasmonic nanomaterials can be expected to be complex and will depend highly on the quantity that is internalized as well as on the amount of energy absorbed and subsequently dissipated by those internalized materials*", the article aims to dissect the initial response of mouse embryonic fibroblasts (MEF) treated with NPRs to a low energy NIR laser irradiation. Knowing from a previous work that nanoprisms irradiated with a higher laser intensity (30 W/cm^2) induce immediately cell necrosis (8), while the same NPRs irradiated at a lower intensity (3W/cm^2) may induce apoptosis in a whole organism (9), the authors push toward major advances in dissecting the cell response to lower energy laser irradiation. Indeed, while maintaining the therapeutic effects, the possibility to employ a laser operating at low energy levels, able to elicit a programmed cell death pathway rather that a fast cell destruction, would be clinically safer. Compared with necrosis, apoptosis is more suitable to *in vivo* treatment since inflammation and even secondary effects associated with necrosis can be avoided. de la Fuente and co-workers address firstly the kinetics and than the mechanisms of cell death by several approaches. To study the kinetics MEF cells were incubated with glucose-modified NPRs (0.1 mg/mL) overnight and then irradiated by an unfocused continuous wave laser at 1064 nm for 30 s, 2, 4, or 10 min. Annexin V (AnnV) and 7-Aminoactinomycin D (7AAD) staining performed at different time points (from 1 h up to 18 h post irradiation), followed by FACS analysis, showed that cells become first apoptotic and only later acquire the secondary necrosis phenotype, i.e., membrane disruption. The production of the heat shock protein Hsp70 early post irradiation further confirms the apoptosis induction, together with assessment of caspase-3 activity at the same time points (2 and 4 min). Altogether these evidences show that the extent of cell death can be regulated simply by modulating the time of irradiation and that the mechanism of cell death may be largely influenced by the intensity of irradiation.

In order to further investigate the mechanism of apoptosis, i.e., either extrinsic or intrinsic pathway, the authors employed MEF cells impaired in the expression of key apoptosis factors, such as the pro-apoptotic proteins of the Bcl-2 family Bak-Bax and Bid or the caspases, (caspase 9 and caspase 3). These cells, under the same experimental condition above described (NPRs 0.1 mg/mL, 2 and 4 min irradiation, 5 h recovery) were shown resistant to apoptosis. The absence of mitochondrial damage (typical of apoptosis), shown by mitochondrial membrane potential measurement in the mutant cell lines, further supported the involvement of mitochondria in cell death execution induced by NPRs. Searching for proteins activating the intrinsic mitochondrial pathway of apoptosis, the authors identify Bid protein as the main player, as shown by the enhancement of the active truncated form tBid after 2 min of irradiation. The mechanism by which Bid was activated remains, however, unresolved, as the caspase-8 inhibitor, IETD, failed to protect cells from NPR-induced photothermal therapy, thereby excluding caspase 8 as the upstream executor of Bid activation and cell death. Trying to address this issue, the authors hypothesize that lysosomal cathepsins released in the cytosol due to disruption of the lysosomes containing NPRs after photothermal treatment might cleave Bid as previously found for other stimuli.

By mean of both immunocytochemistry and transmission electron microscopy performed on MEF treated with NPRs they indeed show the presence of nanoprisms into lysosomes (before irradiation) and 1 h following irradiation their release into the cytoplasm, supporting their hypothesis.

Although a conclusive demonstration of the lysosome involvement in this induced cell death is still pending, due to the lack of efficacy of the cathepsin inhibitors, this interesting work "*want to emphasize the importance of ascertaining the mechanism of cell death induced by hyperthermia, not only to prevent secondary effects such as inflammation but also in order to avoid unsuccessful treatments*". The results and conclusions published by de la Fuente and coworkers may help designing of biocompatible, specific and efficient nanoheaters, planning novel safe therapies and optimizing efficacy of existing photothermal based treatments.

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Footnote

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