Nanoparticle-based dosed photo-thermal therapy with hollow Au/Ag nanoshells amplifies immunogenicity and NK cell cytotoxicity against triple negative breast cancer cells *in vitro*

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Nanoparticle-based photothermal therapy is a powerful and reliable tool used by cancer biologists to induce cell death. One of the most relevant applications in the field is represented by the use of plasmonic gold nanoparticles for cancer-cell targeted ablation mainly characterized for inducing localized heat damage (e.g., intracellular protein coagulation, vapor nanobubble generation or membrane rupture), usually leading to cell death. As a tool, nanoparticle-based photothermal therapy can be efficiently “dosed” via light irradiation, allowing a precise shaping of cell death mechanics after differential light-dependent intracellular content release and apoptotic/necrotic diversification in target cells. Most often, cancer biologists pursue irreparable cell damage in cancer treatments, however, in most translatable scenarios, cellular acute stress response can sustain nanoparticle-based photothermal damage, evade cell death, and generate long-lasting “stress-induced” changes in target cells. These changes may include overexpression of heat shock factors, unfolded protein response elements, as well as membrane stress induced ligands; these ligands are rarely detectable on the surface of healthy cells, but are frequently expressed by transformed, infected or stressed cells. In humans, most of stress-induced ligands are recognized by a single receptor, the natural killer group 2 member D (NKG2D) receptor, majorly expressed by Natural Killer cells [1]. NK cells are cytotoxic lymphocytes, key regulators of innate and adaptive immunities, usually targeting cells with downregulated self-molecules (e.g., HLA class I molecules) or upregulated stress NKG2D ligands (NKG2DL), without prior immunization. Stress ligand induction in cancer cells during treatment is also key feature for cancer immunotherapy in the context of immunogenic cell death, especially when cancer-cell sensitization is a therapeutic option (e.g., localized solid-tumours) [2]. For this reason, in this work we describe the immunogenic potential and stress ligand induction of dosed photothermal therapy with hollow Au/Ag nanoshells in triple negative breast cancer cell lines. For these experiments, we developed a room-temperature method to obtain sterile hollow gold Au/Ag nanoshells via galvanic replacement reactions on spheroid silver nanoparticles, obtained by silver nitrate reduction with ascorbic acid in excess trisodium citrate. Obtained NIR-plasmonic (800 nm) hollow gold/silver nanoshells with average 80-nm diameters and 20-nm shell thickness (TEM) were PEGylated with SH-PEG-NH_2 (3400 Da) and resuspended in PBS prior biological evaluation. First, we typified the cell death mechanism induced by hollow Au/Ag nanoshells in triple negative breast cancer cells (HCC70 & HCC1937) via flow cytometry using Zombie NIR as intracellular/DNA probe and Annexin V as putative apoptotic marker; we comprehensibly validated these results with biochemical-based cell death assays CCK8, MTT and LDH release. Then, we evaluated the immunogenic features of dosed photothermal therapy with hollow Au/Ag nanoshells in triple negative breast cancer cell lines in the context of THP-1 derived macrophages polarization, M1 macrophage phagocytosis, and cytokine production (IL-1β and TNF-α). Finally, we validated NKG2DL induction in triple negative breast cancer cells after dosed photothermal therapy by blocking NKG2D receptor in effector NK-92® co-cultured with treated and non-treated cells. Our preliminary findings show: 1. A photothermal dose-dependent immunogenic response in treated triple negative breast cancer cells, characterized by an increase in necrotic/apoptotic ratios majorly driven by membrane damage/rupture; 2. Triple negative breast cancer cell death (necrotic or apoptotic) was not able to polarize THP-1 monocytes, however it provided a significant boost to phagocytic activity and cytokine release in M1 polarized macrophages; 3. Lower necrotic/apoptotic ratios induced increased IL-1β secretion; 4. Low dose photothermal therapy increased NKG2DL expression in triple negative breast cancer cells and their susceptibility to NK cell lysis (4h).

References

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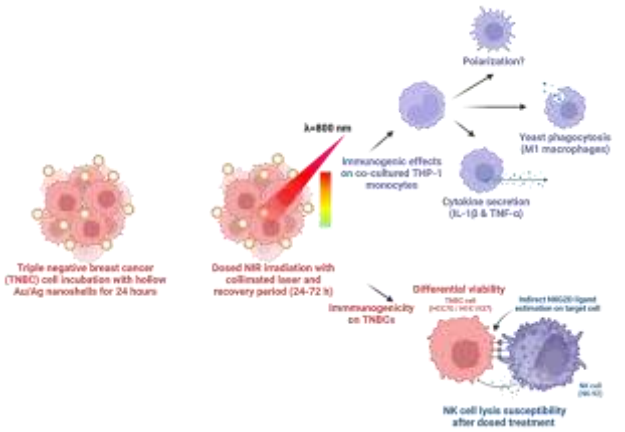

Figure 1. Schematics of the experimental approach. TNBC cells were incubated with hollow Au/Ag nanoshells for 24 h, irradiated with 800 nm laser and let to recover for 24 h. The immunogenic effects of PTT-treated and non-treated TNBC cells on THP-1 monocytes and NK-92 cells were evaluated in vitro.