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Hafnium oxide nanoparticles with radiotherapy induce immunogenic cell death

Short Title:
HfO2 and immunogenic cell death

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Abstract:
Background - Between 70 to 90% of patient have "cold" tumors, i.e. devoid or poorly infiltrated by immune cells, rendering inoperative their treatment by immune checkpoint inhibitors. To allow these patients to benefit from these therapies, it is fundamental to prime an antitumor immune response. Radiotherapy (RT) has demonstrated its ability to induce the immunogenic cell death (ICD), a crucial event allowing the priming of the antitumor immune response. Meanwhile, a new class of material with high electron density, hafnium oxide, was designed at the nanoscale (HfO_2-NP) to efficiently absorb ionizing radiation and increase the radiation dose deposition from within the tumor cells and increase killing of cancer cells. Here, we compared the ability of HfO_2-NP and RT to RT alone to kill cancer cells and induce immunogenic cell death.

Methods - A panel of human and mouse cancer cell lines (mesenchymal and epithelial origin, radiosensitive and radioresistant) were treated or not with HfO_2-NP, then irradiated by X-rays. Impact of the treatments on apoptosis and necrosis was assessed by FACS analysis (Annexin V/Propidium iodide). In addition, the production of the DAMPs characteristic of the ICD (secreted adenosine triphosphate (ATP), ecto-calreticulin (ecto-CALR), and extracellular High Mobility Group Box 1 (HMGB1)) and of two additional DAMPs (ecto-heat shock protein 70 (ecto-HSP70) and 90 (ecto-HSP90)) were determined. The ENLITEN ATP Assay system was used to measure the secreted-ATP. Ecto-(CRT, HSP70 and HSP90) were assessed by FACS analysis and HMGB1 by ELISA assay.

Results - For all the tested cell lines treated with HfO_2-NP and RT, a marked increase of apoptosis and necrosis was demonstrated, compared to cells treated with RT alone. In addition, higher levels of DAMPs (ecto-CRT, ecto-HSP70, ecto-HSP90, secreted ATP and extracellular HMGB1) were measured in the cancer cells treated with HfO_2-NP and RT when compared to cancer cells exposed to RT.

Conclusion - HfO_2-NP has demonstrated its capacity to kill cancer cells more efficiently than radiotherapy alone. HfO_2-NP, administered via a single intratumor injection, is currently evaluated in clinical trials including soft tissue sarcoma (phase II/III), head and neck, prostate, liver and rectum cancers (phase I) and would permit to improve the local control of tumors, a crucial parameter for the cure and survival of patients. Here, we further show that the superior ability of HfO_2-NP and RT treatment to generate ICD would prime an antitumor immune response with more effectiveness than RT alone can do, converting the tumor into an actual *in situ* vaccine. Thus, this transformation by HfO_2-NP of immunologically “cold” tumors into “hot” tumors would open a new avenue for the use of immune checkpoint inhibitors across oncology, particularly for non- and poor-responder patients.