TITLE

Transforming immunologically "cold" tumor into "hot" tumor with hafnium oxide nanoparticles and radiation therapy

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Background

Hafnium oxide, an electron-dense material, was designed at the nanoscale to increase the radiation dose deposited from within the cancer cells: "Hot spot" of energy deposit where the nanoparticles are when exposed to radiation therapy (RT). Preclinical studies have demonstrated increase of cancer cells killing *in vitro* and marked antitumor efficacy *in vivo* with presence of these nanoparticles (HfO_2-NP) exposed to RT, when compared to RT alone. HfO_2-NP is intended for a single intratumor injection and is currently evaluated in clinical trials including soft tissue sarcoma, head and neck, prostate, liver and rectum cancers.

Here, we explore the ability of nanosized hafnium oxide exposed to RT to bring substantial immune cells infiltrations in the tumors and convert immunologically "cold" tumor into "hot" tumor.

Materials and methods

CT26 (murine colorectal cancer cells) were subcutaneously injected in the flank of BALB/c mice. Once the mean tumors volume reached 115±30 mm^3, tumors were intratumor injected with HfO_2-NP and irradiated with 2Gyx3 or 4Gyx3, or irradiated only. Tumors were collected 5 days after the last RT fraction and analyzed for immune cell infiltrates by immunohistochemistry (2Gyx3 and 4Gyx3) and cytokines content by flow cytometry (2Gyx3).

A second study evaluated HfO_2-NP exposed to RT *vs* RT alone using the 4T1 murine breast cancer model. Cells treated or not with HfO_2-NP were exposed to irradiation (40Gy). Irradiated cells (1.10^6) (or phosphate-buffered saline as control) were inoculated subcutaneously into the flank of BALB/c mice (vaccination phase). Seven days after, mice were challenged with untreated 4T1 cells (1.10^6) (challenge phase). Grown tumors (challenge site) were collected 19 days after the challenge phase and analyzed for immune cell infiltrates by immunohistochemistry.

Results

In mice bearing CT26 tumors, a marked increase of cytokines content and immune cell infiltrates was observed with HfO_2-NP + 2Gyx3 when compared to RT alone. The tumor immune cell infiltrates were further enhanced with HfO_2-NP + 4Gyx3.

In mice inoculated with 4T1 cells treated with HfO_2-NP + 40Gy, a marked increase of immune cell infiltrate (CD8+) was observed in tumors when compared to tumors in mice inoculated with 4T1 cells treated with 40Gy and control.

Conclusions

These *in vivo* data generated from CT26 and 4T1 tumor models suggest that HfO_2-NP + RT triggers immunogenic conversion of the tumor microenvironement when compared to RT alone. HfO_2-NP treatment may represent a therapeutical approach for broad applications since it does not rely on any molecular characteristics of the tumor.