Cancer immunotherapy based on MUSIC platform and STING activation in brain cancer cells

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Background

Cancer immunotherapy has shown promise as a treatment that aids patients' immune systems to recognize and respond to cancer. Immunotherapy works by overriding immune system checkpoints to attack cancer cells. Immune checkpoints downregulate adaptive immune responses, typically preventing them from becoming too strong. Cancer cells usurp these checkpoints to evade T cells and avoid elimination. By blocking these checkpoints, immunotherapy enables T cells to effectively kill cancer cells. However, only a small percentage of patients who undergo immunotherapy, such as T cell checkpoint blockade, respond favorably to treatment. To develop a more effective immunotherapy strategy that benefits a greater number of cancer patients, there has been a growing realization that stimulation of both the innate and adaptive branches of the body's immune system is necessary to generate systemic antitumor immunity.

With the advancement of new immunotherapies targeting regulators of the innate immune system, MD Anderson Cancer Center researchers of Drs. Kim and Jiang pioneered the Microbubble-assisted UltraSound-guided Immunotherapy of Cancer (MUSIC) strategy that targeted and efficient immune activation while maximizing antitumour effects and minimizing toxicity. (Li, 2022, 898). This platform uses nanocomplex-conjugated microbubbles (ncMBs) that specifically target antigen-presenting cells (APCs) to effectively deliver cGAMP into their cytosol, facilitated by ultrasound (US)-guided release, to activate the Stimulator of Interferon Genes (STING) pathway. The activation of STING stimulates type I interferon (IFN) responses, which are crucial for priming tumor-specific cytotoxic T cells. This innovative use of immunotherapy allows for targeted and efficient activation of STING with cGAMP and provides efficient priming of antigen-specific T cells in primary tumors.

While the MUSIC platform has been successful in breast cancer, there is an interest in demonstrating the versatility of the MUSIC platform with other cancer types, specifically brain cancer.

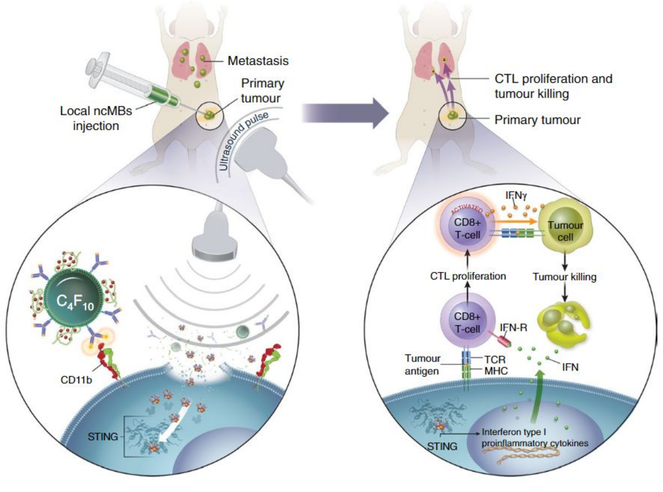
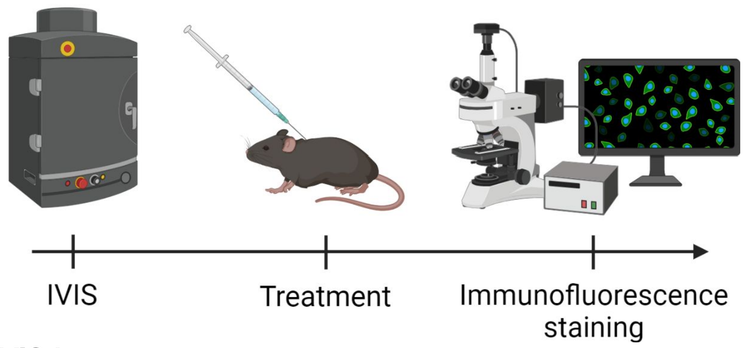


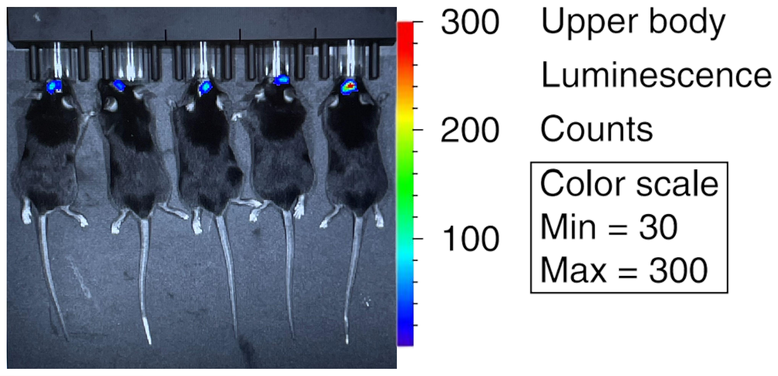
Figure 1. Nanocomplex-decorated microbubbles targeting CD11b on APCs. On binding of ncMBs to APCs and under US exposure, cGAMP is delivered directly into the cytosol of the APCs by sonoporation to activate STING and downstream antitumour immunity, a process termed MUSIC.

Methods



IVIS images

To monitor the progression of the tumor in the mice, we used IVIS imaging and luciferin. Luminescence was measured with an in vivo imaging system to evaluate the luminescence intensity. Luciferin was injected into each mouse. Luciferin is a common bioluminescent reporter for in vivo imaging of expressing luciferase (oxidative enzymes that produce bioluminescence). The reaction between the luciferin substrate paired with the receptor enzyme luciferase produces a catalytic reaction, generating bioluminescence. The IVIS images of the mice's heads were used to detect luminescence signals in the brain.



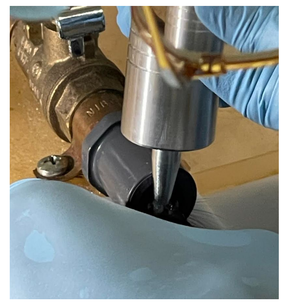
MUSIC treatment

Four different groups of mice were treated: 1) cGAS (cGAMP only), 2) Empty microbubbles with ultrasound, 3) MUSIC (cGAMP filled microbubbles with ultrasound), 4) no treatment (only injected tumour). Five mice in each group which were a) anesthetized with isoflurane, b) then injected with 5 µL of treatment near the bolt, and one group was treated with ultrasound. Lastly, the incisions made onto the mice are closed.

a)

b)

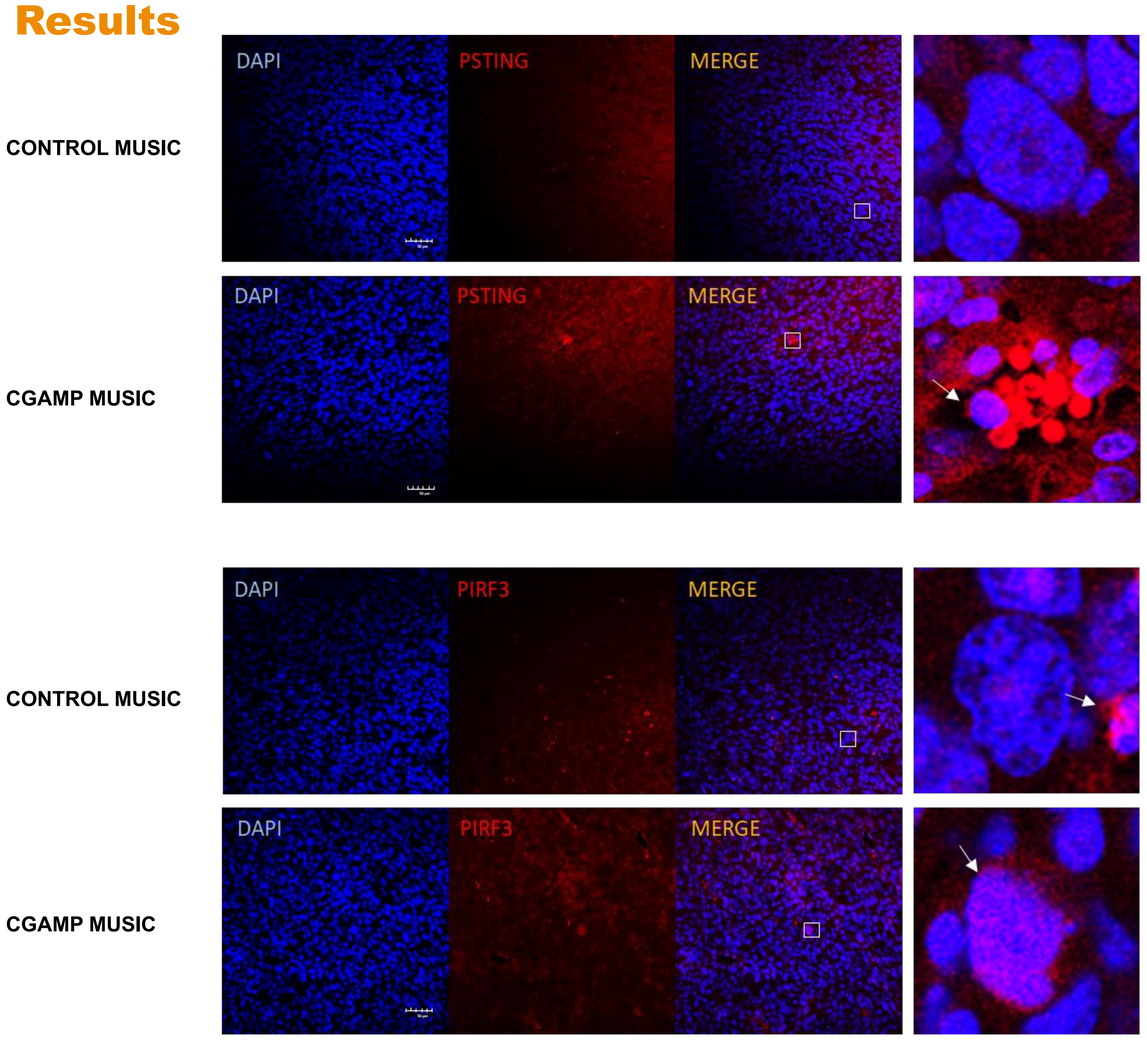
c)



Immunofluorescence staining

We stained four sets of slides that were controlled, treated with cGAMP and MUSIC. We baked slides in 60°C incubator for 30 mins, then deparaffinized with xylene, washed with ethanol, and blocked with 10% goat serum + 90% TBST + 2% BSA + 0.3% Triton x100. After adding the blocking buffer, incubate for 2 hours. Primary antibody solutions PSTING and PIRF3 (1-200 µL dilution) were added to incubate overnight in the dark at 4°C. After washing the primary solution, secondary antibody Alexa Fluor 546 (1-2000 µL dilution) was added then incubated in room temp for 1 hour. After adding DAPI stain (1-1000 µL dilution), incubate for 8 mins at room temp. After adding autoquench solution (1-20 µL dilution), incubate for 2 mins. We used these two antibodies and DAPI to image signals of either PSTING or PIRF3 being activated.

Results



Conclusions

Based on the results of the images, the cGAMP MUSIC treatment was effective. In the confocal fluorescence microscopy images, we can see higher fluorescence intensity and activation in PSTING and PIRF3 ((phosphorylated IRF3). The MUSIC treatment produced increased phosphorylation of STING and IFN regulatory factor 3 (IRF3) in the mice brain tumor cells. We can hypothesize that the MUSIC treatment is effective in brain cancer since in the reference paper demonstrated that MUSIC can effectively enhance STING activation in APCs, leading to improved priming of T cell responses.

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References

Li, X., et al., (2022). Cancer immunotherapy based on image-guided Sting activation by nucleotide nanocomplex-decorated ultrasound microbubbles. Nature Nanotechnology, 17(8), 891–899. https://doi.org/10.1038/s41565-022-01134-z