Engineered Nanobubbles for Inducing Unprogrammed Necrosis in Cancer Immunotherapy

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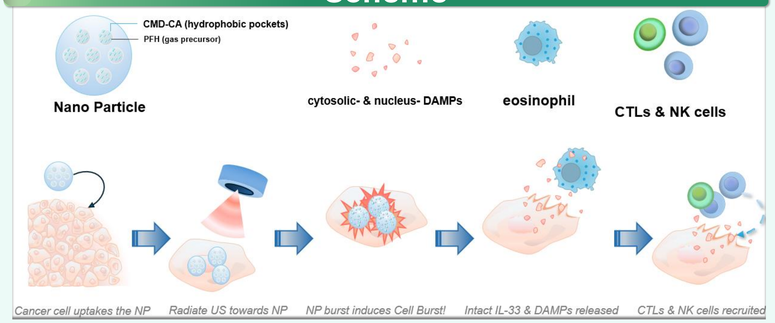
KEY WORDS: *IL-33, eosinophils, unprogrammed cell death, CTLs and NK cells recruitment*

Abstract

Unprogrammed cell deaths play a key role in the modulation of inflammatory responses by releasing various immunostimulatory molecules. In particular, accidental necrosis can activate eosinophils by releasing interleukin-33 (IL-33) and thereby provide an exceptional opportunity for the recruitment of natural killer cells and cytotoxic T-cells in the antitumor immune response. In this research, we designed nanobubbles that are cavitated by ultrasound to induce cell death, which also dramatically leaks IL-33 and damage-associated molecular patterns (DAMPs).

Scheme



Conclusion

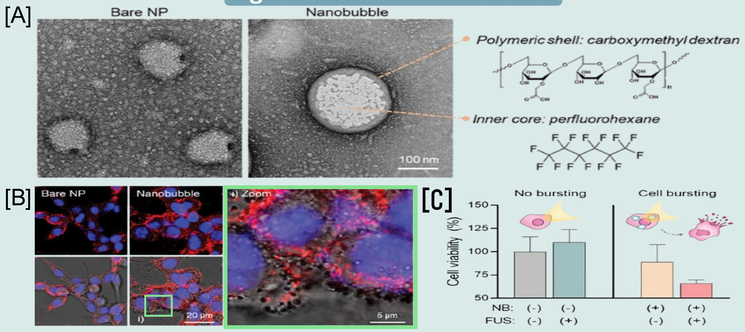
NanoBurst triggers complete **IL-33 & DAMP release** via ultrasound-induced cell rupture
Released DAMPs → Eosinophil activation
→ **Robust CTL/NK cell recruitment** at tumor site
with DPP4i combination shows [CD69$^+$ (2.66×) ↑ , CD8$^+$ (1.46×) ↑] in lung
(Stronger than NanoBurst + aPD-1)

∴ NanoBurst-driven CTL/NK recruitment fuels potent antitumor immunity!

SCAN ME
References!

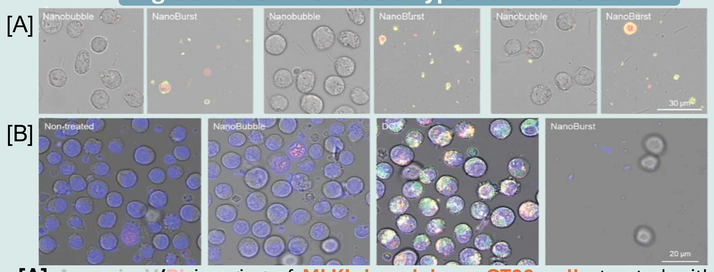
Results

Fig.1 Characterization of NPs



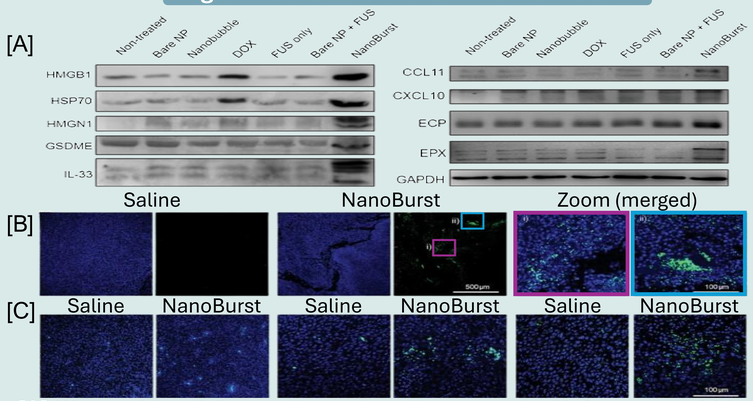
[A] TEM images and the **chemical structures** of nanobubble components. **[B]** Confocal microscopic images of **CT26 cells** treated with **(left)** bare NPs or **(right)** nanobubbles (100μg/mL) for 6h. **(DAPI & Cy5.5)** **[C]** **Cell viability** of CT26 cells treated with ultrasound, **(left)** nanobubbles, or **(right)** NanoBurst.

Fig.2 Different cell death type from Doxorubicin



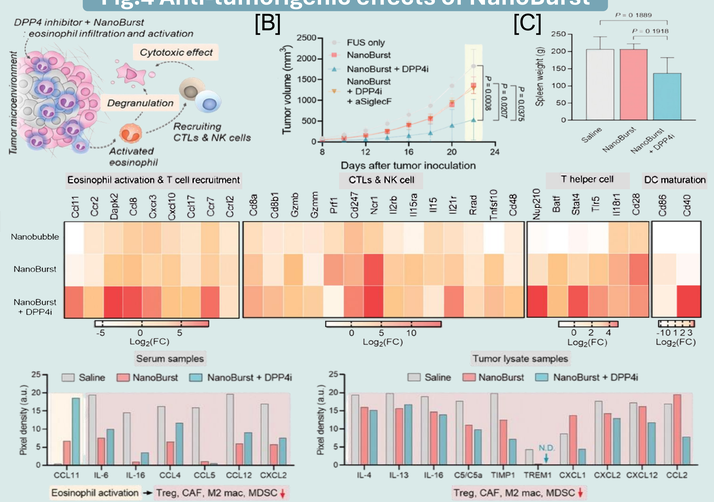
[A] Annexin V/PI imaging of **MLKL knockdown CT26 cells** treated with **(left)** nanobubbles or **(right)** NanoBurst. **[B]** Caspase-3 imaging (DAPI, DOX, Cas3) of **CT26 cells treated** with [**non-treated I nanobubbles I DOX I NanoBurst**].

Fig.3 Intact DAMP release via NanoBurst



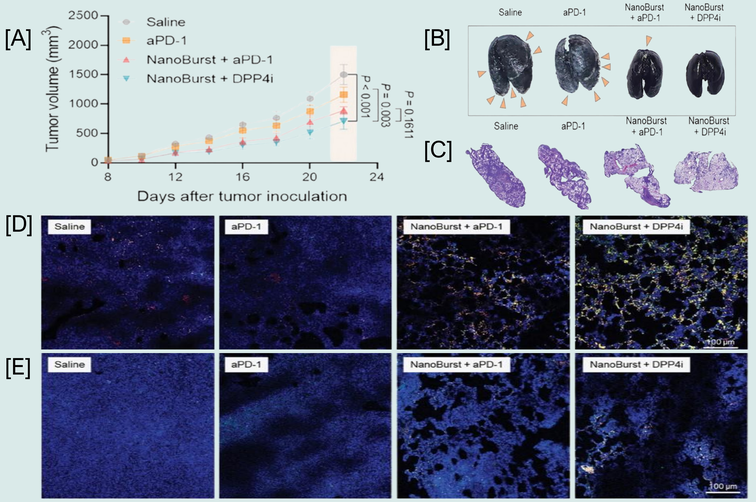
[A] Western blot of the **conditioned medium treated** with [**non-treated I bare NPs I nanobubbles I DOX I US I bare NPs + US I NanoBurst**] **(left)** from CT26 cells, **(right)** from eosinophils after incubation with the CT26 cells. **[B]** Immunohistochemistry of **activated eosinophils** in tumor tissues. **(DAPI & SiglecF)** **[C]** Immunohistochemistry of **DAPI & CTLs, NK cells, and matured DCs** in tumor tissues.

Fig.4 Anti-tumorigenic effects of NanoBurst



[A] Schematics for anti-tumorigenic effects. **[B]** Changes in **tumor volume** for each treatment group (n=4). **[C]** **Weight of the spleens** for each treatment group (Saline, NanoBurst, NanoBurst+DPP4i). **[D]** **Heat map of mRNA levels** in tumor tissues for each treatment group (**Nanobubble / NanoBurst / NanoBurst+DPP4i; downwards**). **[E]** **Cytokine and chemokine levels** for each treatment group (Saline, NanoBurst, NanoBurst+DPP4i).

Fig.5 Synergic effects of NanoBurst + DPP4i



[B]~[E]: [saline I aPD-1 I NanoBurst + aPD-1 I NanoBurst + DPP4i]
[A] The average tumor volume. **[B]** Representative photographs of India-ink-stained **lung tissues**. **[C]** Hematoxylin and eosin staining images of the **lung tissues**. **[D]** Immunohistochemistry of **activated eosinophils**. **(DAPI, SiglecF, CD69)** **[E]** **CTLs** in the lung tissues. **(DAPI, CD3, CD8)**