Enzyme-switchable polymer conjugate induces lysosomal pyroptosis and enhances PD-1 blockade

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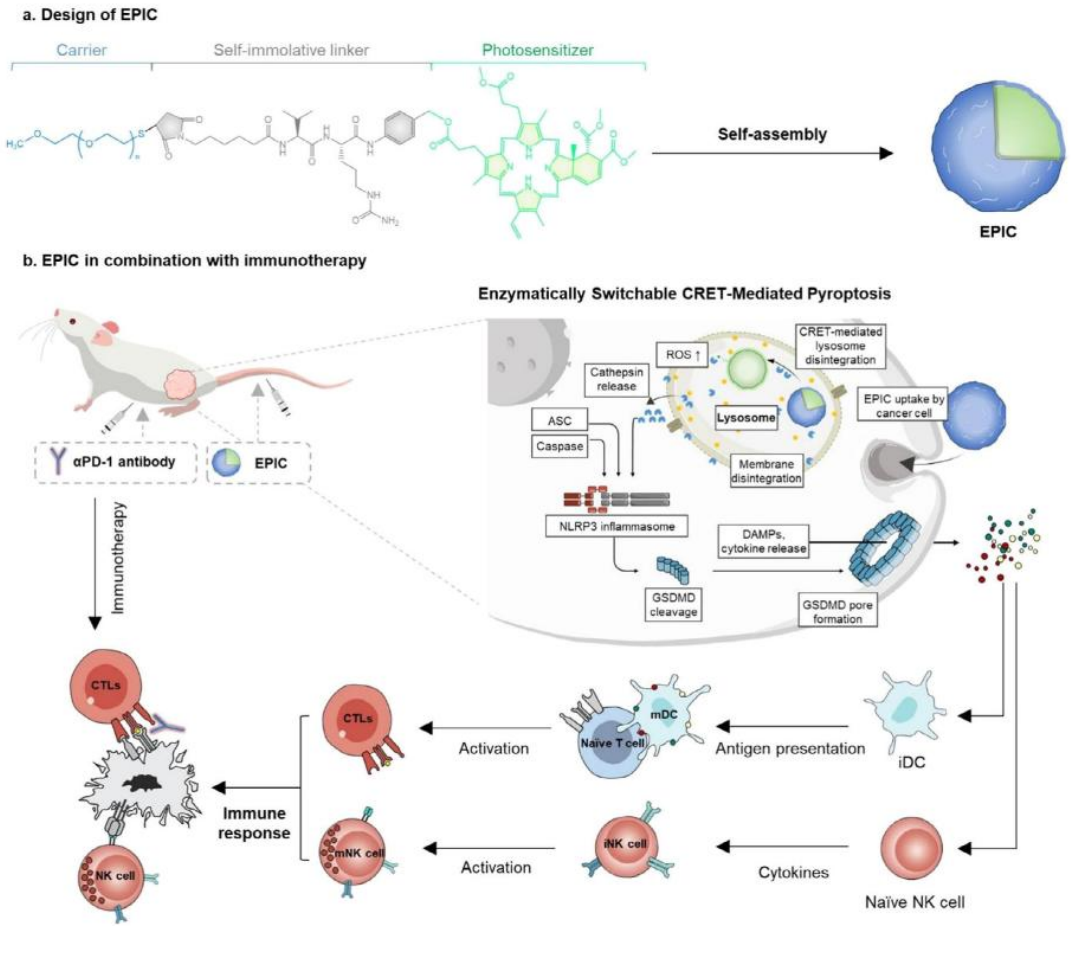
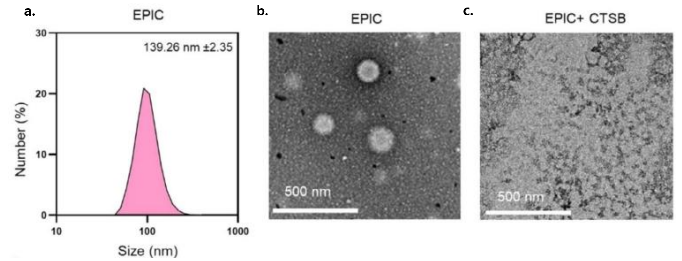
Scheme



Abstract

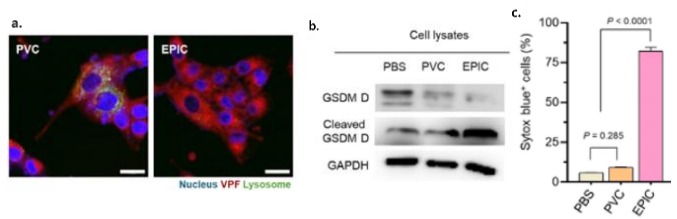
Gasdermin D (GSDMD)-mediated pyroptosis can turn tumor-restricted damage into immune priming, yet spatiotemporal activation of GSDMD is not acheived. Here we describe an enzymatically switchable pyroptosis-inducing polymer conjugate (EPIC) that triggers lysosome-confined chemiluminescence resonance energy transfer (CRET) in response to cathepsin B (CTSB), a protease enriched in many cancer cells. EPIC is an amphiphilic PEG–verteporfin (VPF) conjugate incorporating a CTSB-cleavable valine-citrulline (Val–Cit) self-immolative linker, enabling nanoparticle self-assembly and intracellular activation. After lysosomal CTSB cleavage, CRET activates VPF without external irradiation, generating reactive oxygen species that destabilize lysosomal membranes and promote CTSB escape into the cytosol. This initiates NLRP3 inflammasome signaling, GSDMD cleavage, and pore formation, leading to membrane rupture with release of damage-associated molecular patterns and inflammatory cytokines. In 4T1 cells, EPIC induced lysosomal disruption, robust GSDMD cleavage, Sytox-positive membrane damage, and release of HSP70 and IL-1β, while conditioned media promoted maturation of bone marrow–derived dendritic cells. Systemic administration in 4T1 tumor–bearing mice increased intratumoral NLRP3 and IL-1β/IL-18 and enhanced innate and adaptive immune activation, including enhancement of NK cell function. Cathepsin B–dependent lysosomal activation of EPIC induced localized pyroptosis and inflammatory signaling, leading to improved antitumor immunity and enhanced checkpoint therapy.

Characterization



Characterization of EPIC
a) Hydrodynamic size distribution of EPIC measured using DLS.
b) Representative TEM image of EPIC. Scale bar, 500 nm.
c) Representative TEMimage of EPIC after 24 h of incubation with CTSB in MES buffer. Scale bar, 500 nm

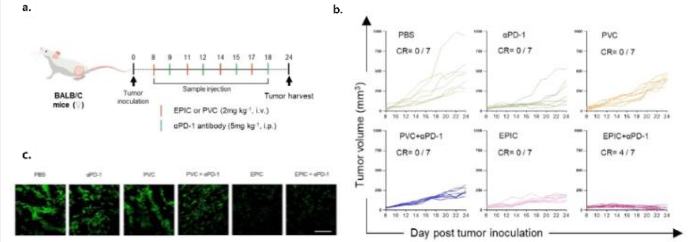
Result : *In vitro*



EPIC induces immunogenic pyroptosis

a) Representative confocal fluorescence microscopy imaging of EPIC-mediated,CTSB-responsive lysosomal destabilization. Scale bars, 20 μm.
b) Western blot assay to examine pyroptosis-associated proteins in the cell lysate (GS-DMD, cleaved GSDMD).
c) Portionof Sytox blue-stained 4T1 cells after 6 h incubation with PVC or EPIC. n = 3 biological replicates. The error bars represent mean ± S.E.M.

Result : *In vivo*



Treatment with EPIC promotes tumor regression in a 4T1 breast cancer mouse model by inducing pyroptosis.

a) 4T1 tumor-bearing Balb/cmice were treated with PVC or EPIC, αPD-1, or combinations there of on selected days post-inoculation.
b) Tumor volumes were measured regularly post-inoculation. n=7 biological replicates.
c) Representativeconfocal fluorescence microscopy imaging of GSDMD protein expression in tumor tissues. Scale bars, 100 μm.

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