Celastrol-Cu Nanoparticles Induce Self-Amplified Cuproptosis Augmented Cancer Immunotherapy

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Introduction

Cuproptosis, a recently identified copper-dependent programmed cell death pathway, has emerged as a promising strategy in cancer therapy. Its effectiveness is closely linked to intracellular copper accumulation but is mitigated by elevated levels of glutathione (GSH) in tumor cells. A significant barrier to its clinical application lies in the challenge of facilitating the cellular uptake of copper ions. Additionally, intracellular copper ions rapidly bind with GSH, further complicating the induction of tumor cell death. Notably, no existing ionophore drug simultaneously acts as both a copper ionophore and a GSH scavenger to effectively induce cuproptosis.

Material and Methods

Celastrol (Cel), a compound extracted from Tripterygium wilfordii, demonstrates diverse biological activities. Its molecular structure includes C-2 carbonyl and C-3 hydroxyl groups, which enable the chelation of metal ions. Furthermore, Cel inhibits GSH synthesis by suppressing the nuclear factor kappa-B (NF-⊠B) pathway. To develop safe and effective bifunctional therapeutic agents, we synthesized celastrol-copper complexes (Cel-Cu) by leveraging the C-2 carbonyl and C-3 hydroxyl groups of Cel to form coordination bonds with copper ions. These complexes were further self-assembled with DSPE-PEG2000 to create nanoparticles (Cel-Cu NP). Comprehensive characterization of both the complexes and the nanomedicine was conducted, followed by systematic evaluation of their in vitro anti-tumor activity, mechanisms of action, and in vivo therapeutic efficacy in animal models.

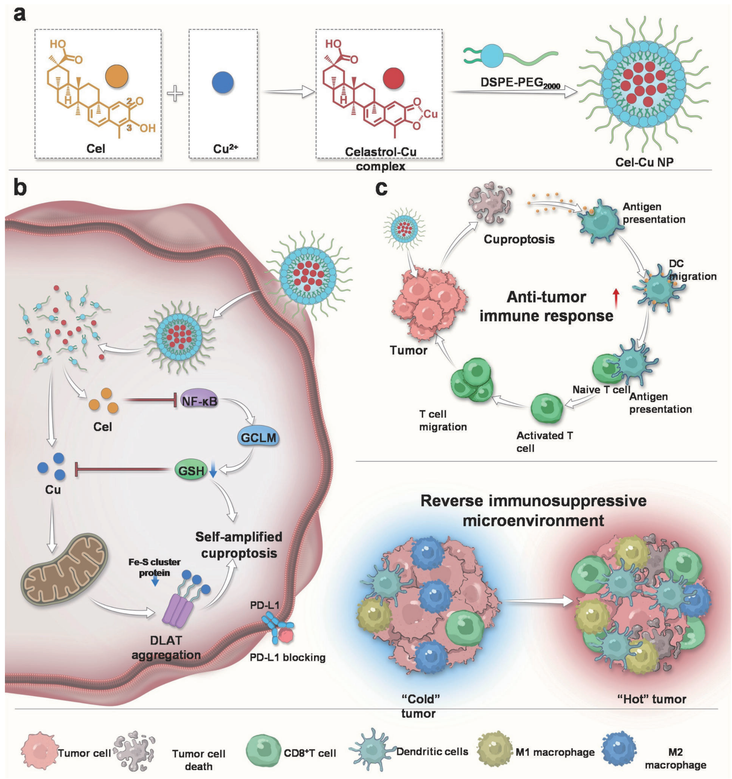

Figure 1: Preparation of Cel-Cu NP for self-amplified cuproptosis

a) Schematic illustration showing the preparation of Cel-Cu NP. b) The biological mechanism of self-amplified cuproptosis induced by Cel-Cu NP. Upon cell internalization, Cel-Cu NP can simultaneously release copper and Cel in tumor cells. The released copper bind to lipoylated dihydrolipoamide S-acetyltransferase (DLAT), hence triggering cuproptosis. Meanwhile, the released Cel inhibits the nuclear factor kappa-B (NF-κB) pathway to scavenge glutathione (GSH) in tumor cells, which enhances the efficacy of cuproptosis in a self-amplified manner. c) The self-amplified cuproptosis further induces immunogenic cell death (ICD) to reverse immunosuppressive tumor microenvironment, which augments cancer immunotherapy.

Results 1

- The carboxyl and hydroxyl groups located at the C2-C3 positions of Cel are capable of binding with copper ions.
- Cel-Cu was encapsulated via DSPE-PEG2000 to form polymeric nanoparticles

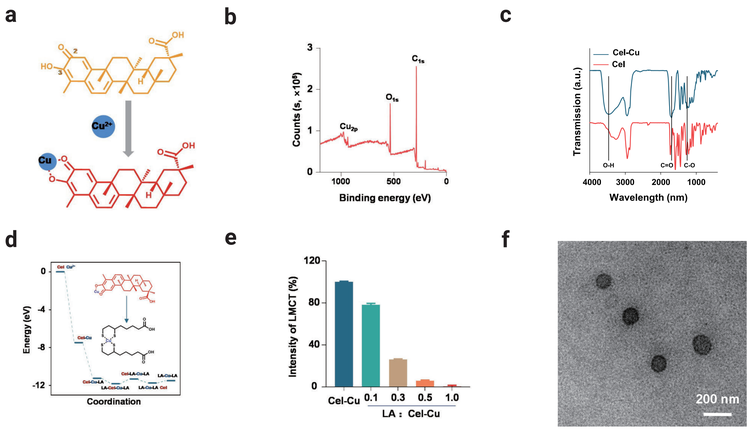

Figure 2: Preparation and characterization of Cel-Cu complex and Cel-Cu NP.

a) Schematic showing the coordination of Cel and Cu2+ to form Cel-Cu. b) X-ray photoelectron spectroscopy (XPS) survey of Cel-Cu. c) Fourier transform infrared spectroscopy (FTIR) spectra of Cel and Cel-Cu. d) Schematic diagram of the binding energy for Cel-Cu calculated by density functional theory (DFT). e) The ligand-to-metal charge transfer (LMCT) intensity of the Cel-Cu solution (200 μm) lipoic acid at 550 nmwith increasing molar ratios of. The inserted graph represents the color changes in the samples. f) TEM images of Cel-Cu NP.

Results 2

- Cel-Cu NP exhibits a superior effect in killing cells.
- Cel-Cu NP scavenge intracellular GSH to amplify cuproptosis.
- Cel-Cu NP induced-cuproptosis evokes immunogenic cell death (ICD) to activate immune response.

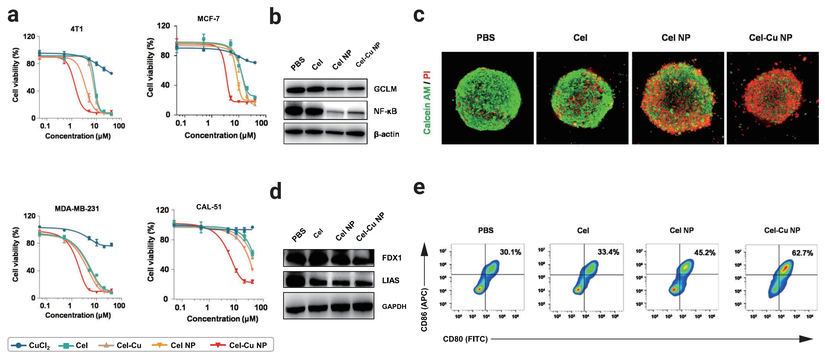

Figure 3: In vitro evaluation of the anti-cancer effect of Cel-Cu NP.

a) Cell viability of 4T1, MCF-7, MDA-MB-231, and CAL-51 cells after various treatments.b) Protein expressions of NF-κB), GCLM, and actin in 4T1 cells determined by Western blot. c) Representative CLSM images of 4T1 cells multicellular tumor spheroids (MCTS) upon various treatments and live (green)/dead (red) staining. d) Protein expressions of FDX1, LIAS, and GAPDH in 4T1 cells determined by Western blot upon various treatments. e) flow cytometry plot of maturation of BMDCs in 4T1 cells upon various treatments. Data are represented as mean ± SD. p values were calculated via one-way ANOVA test in d), f), and g). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant.

Results 3

- Cel-Cu NP could effectively accumulate in the tumor site and induce the maturation of dendritic cells.
- Combination Therapy of Cel-Cu NP and αPD-L1 demonstrate superior effects in the 4T1 metastasis breast cancer model

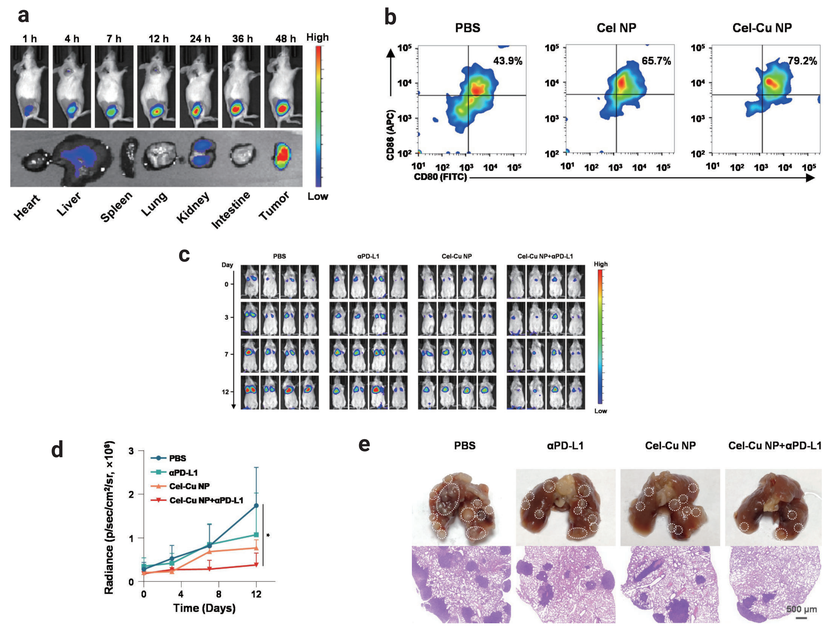

Figure 4: In vivo evaluation of the anti-cancer effect of Cel-Cu NP.

a) In vivo biodistribution of Cel-Cu NP labeled with Cy5.5 using an in vivo imaging system (IVIS). b) Flow cytometric analysis of dendritic cells (DCs) in tumor tissues of mice upon various treatments. c) Growth of 4T1-LUC tumors in mice visualized by IVIS. d) Quantification of the luminescence signal from c. e) Photographs of metastatic nodules (highlighted write circles) in the lungs and hematoxylin and eosin (H&E) staining of the organ slices obtained from mice upon various treatments.

Conclusions

A celastrol-based nanomedicine has been developed. Cel-Cu NP effectively promote cuproptosis in a self-amplifying manner, as validated in both 4T1 subcutaneous and metastatic breast cancer models. This Cel-Cu NP platform provides a novel option with significant translational potential for the integrated programmed therapy of triple-negative breast cancer (TNBC) in immunologically "cold" tumors.

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