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Perspective

Calcium ion nanomodulators for mitochondria-targeted multimodal cancer therapy

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Various calcium ion (Ca^{2+}) nanomodulators are designed for multimodal cancer treatment with the mechanism of intramitochondrial Ca^{2+} overload-induced multilevel mitochondrial destruction. This perspective briefly introduces the development of Ca^{2+} nanomodulators in cancer therapy based on two recent studies published by our research group.

Taking advantage of accurate drug delivery and reduced side effects, subcellular organelle-targeted nanoformulations have attracted more and more attention from cancer therapists. As an essential organelle of mammalian cells, mitochondria play a crucial role in energy conversion, tricarboxylic acid cycle, apoptosis, oxidative stress, calcium ion (Ca^{2+}) storage, and so on. Among these, Ca^{2+} storage is an indispensable work. As one of the key second messengers in the cells, Ca^{2+}participates in a wide range of physiological processes, such as the control of biomembrane permeability and cell excitability, cell metabolism, maintenance of cell morphology, cell cycle regulation, and so froth, and is the hub of a variety of cell signal transmission pathways. Typically, bound calcium and free Ca^{2+} are keeping in a dynamic balance. Once the free Ca^{2+} increases sharply under some conditions, the balance of intramitochondrial Ca^{2+} is broken, leading to cell apoptosis. However, the Ca^{2+} signaling pathway in cancer cells is easily changed by certain drugs, making them more sensitive to the increase of Ca^{2+} concentration than that of normal cells. Hence, intramitochondrial Ca^{2+} overload, which disrupts mitochondrial Ca^{2+} homeostasis, may be an effective strategy for precision cancer therapy [1,2].

We recently developed a multichannel Ca^{2+} nanomodulator ($^{PEG}CaNM_{CUR+CDDP}$) to boost Ca^{2+} overload-mediated mitochondrial dysfunction in cancer treatment, as shown in Scheme 1A [3]. A Ca^{2+} enhancer curcumin (CUR), which increased mitochondrial Ca^{2+} level and inhibited Ca^{2+} efflux, and a mitochondrial dysfunction drug cisplatin (CDDP), which induced mitochondrial damage, were co-encapsulated into $^{PEG}CaNM_{CUR+CDDP}$. Comparing to other reported a2+ nanomodulators, PEGCaNMCUR+CDDP have the following advantages: (a) Simple synthesis steps and high drug loading efficiency, (b) tumor microenvironment-triggered gradual release behavior with enhanced therapeutic efficacy and reduced systemic toxicity, (c) realized multilevel mitochondrial damage, and (d) excellent fluorescence and PA imaging capacities.

After intravenous injection, the monodisperse spherical nanoparticle $^{PEG}CaNM_{CUR+CDDP}$ successfully accumulated into the human MCF-7 breast cancer xenograft model through the enhanced permeability and retention (EPR) effect after detachment of poly(ethylene glycol) (PEG) and enhanced cell uptake. A mass of released Ca^{2+} with the assistance of CUR and CDDP achieved multilevel mitochondrial dysfunction. The decreased mitochondrial membrane potential and number of mitochondria, the severest destruction of mitochondrial morphology, the lowest intracellular adenosine triphosphate

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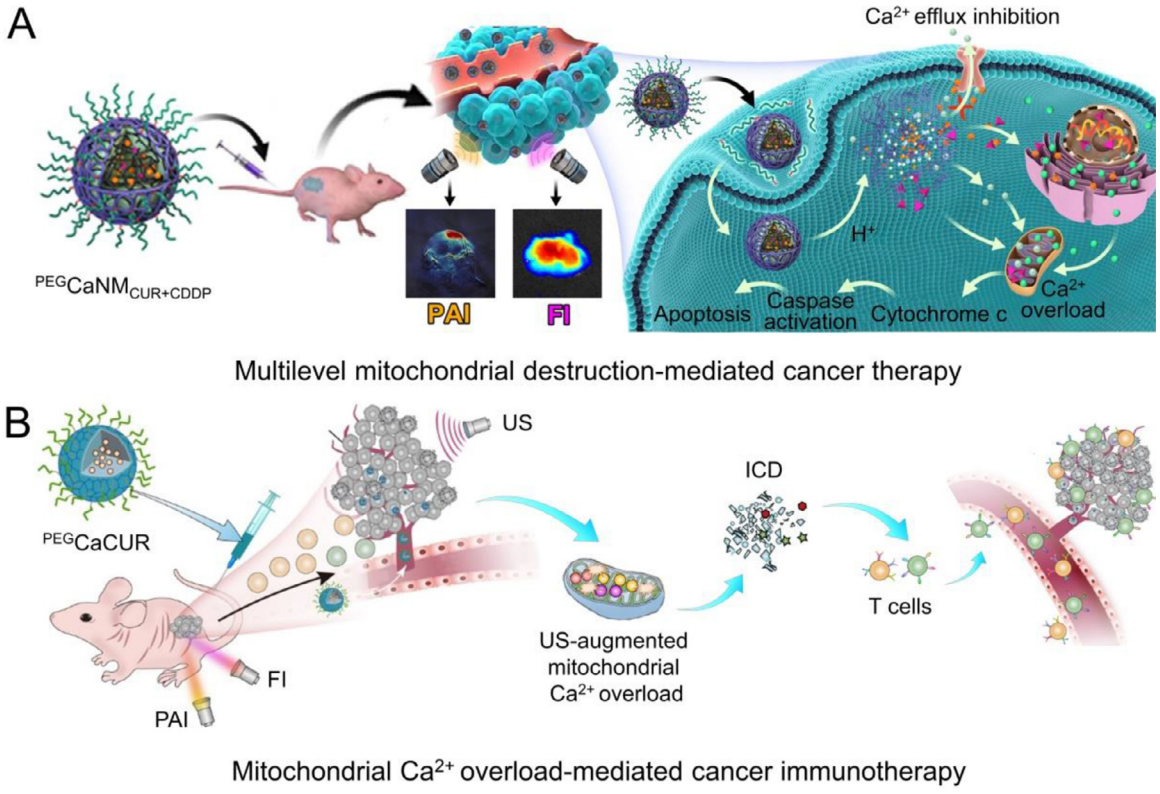

Scheme 1 – Various calcium nanomodulators for mitochondria-targeted multimodel cancer therapy.

(ATP) level, and the highest expression of apoptosis proteins were revealed in the $^{PEG}CaNM_{CUR+CDDP}$ group, and all the results indicated that $^{PEG}CaNM_{CUR+CDDP}$ was an efficient Ca^{2+} nanomodulator for enhanced cancer treatment.

Although Ca^{2+} nanomodulators have been developed for tumor treatment through activating mitochondrial apoptosis pathways *via* mitochondria Ca^{2+} overload, the specific mechanism has not been proven. For the first time, the RNA obtained from $^{PEG}CaNM_{CUR+CDDP}$-treated MCF-7 cells was sequenced. The results showed that the positive correlated genes of the $^{PEG}CaNM_{CUR+CDDP}$ group were mainly in the terms like "positive regulation of cell death", "cellular carbohydrate metabolic process", and "anion transport", while negative correlated genes were primarily in some biological processes like "mitochondrial gene expression", "mitochondrial transport", and "negative regulation of cell cycle". Therefore, $^{PEG}CaNM_{CUR+CDDP}$ could activate mitochondrial apoptosis pathways *via* mitochondria Ca^{2+} overload. In addition, this nanoplatform possessed excellent fluorescence and PA imaging capacities. Hence, the multifunctional Ca^{2+} nanomodulator $^{PEG}CaNM_{CUR+CDDP}$ is a promising organelle-targeted theranostics nanoplatform for cancer treatment.

The successful application of these Ca^{2+} nanomodulators in cancer chemotherapy pushed us to explore whether they can activate antitumor immune or not. Immunogenic cell death (ICD), which can activate antitumor immune responses [4,5], received plenty of focus in cancer immunotherapy [6,7]. Hence, in our published study [8], an acid-sensitive PEG-decorated Ca^{2+} nanomodulator ($^{PEG}CaCUR$) immunogenic cell death (ICD)-inducing properties, as shown in Scheme 1B. Different from $^{PEG}CaNM_{CUR+CDDP}$, $^{PEG}CaCUR$ was synthesized without PDA and CDDP. After being combined with ultrasound (US), an exogenous physical stimulus, which could upregulate the intracellular Ca^{2+} concentration, $^{PEG}CaCUR$+US led to an enhanced Ca nanomodulator.

The monodisperse, spherical, and amorphous nanoparticles $^{PEG}CaCUR$ released a mass of Ca^{2+} and CUR at intracellular low pH conditions to caused mitochondrial dysfunction through inducing mitochondrial Ca^{2+} overload, featured by lower mitochondrial membrane potential, fewer mitochondria, and more severe destruction of mitochondrial morphology.

For detecting the ICD-inducing properties of $^{PEG}CaCUR$, the levels of calreticulin (CRT), high-mobility group box 1 (HMGB1), and adenosine triphosphate (ATP) were detected. Interestingly, the cells treated with $^{PEG}CaCUR$ showed CRT exposure and elevated release of HMGB1 and ATP. However, more cell-surface CRT exposure and higher extracellular HMGB1 and ATP levels were found in the $^{PEG}CaCUR$+US group. All these results verified that mitochondrial Ca^{2+} overload could induce significant ICD, which we could further improve. Then, we proved that reactive oxygen species (ROS) generated by the mitochondrial Ca^{2+} overload contributed to the happen of ICD, and more ROS generation in the $^{PEG}CaCUR$+US group evoked enhanced ICD efficacy. After six-time treatments, $^{PEG}CaCUR$ exhibited a moderate immune activation effect. As expected, $^{PEG}CaCUR$+US activated more efficient antitumor immune responses, resulting in effectively suppressing tumor growth and metastasis.

Although these two Ca^{2+} nanomodulators exhibited excellent efficacy of tumor therapy, some issues should be further dissolved in the future. The content of Ca^{2+} in $^{PEG}CaNM_{CUR+CDDP}$ and $^{PEG}CaCUR$ were lower compared with the extracellular fluid. Except for the generation of free Ca^{2+} in the cells and inhibition of efflux, inducing a large influx of extracellular Ca^{2+} is an effective way to cause mitochondrial Ca^{2+} overload. Hence, it's urgent to develop a new Ca^{2+} nanomodulator with multifunction for more efficient Ca^{2+} accumulation in mitochondria. In addition, expanding the application range of Ca^{2+} nanomodulator is meaningful.

In summary, the developed Ca^{2+} nanomodulators could efficiently inhibit the progression of cancers by inducing significant mitochondrial Ca2+ overload with multimodal imaging, which could cause the increased level of intracellular ROS and robust ICD, indicating their great potential for the theranostics of cancers in clinic.

Conflicts of interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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REFERENCES

[1] Xu L, Tong G, Song Q, Zhu C, Zhang H, Shi J, et al. Enhanced intracellular Ca^{2+} nanogenerator for tumor-specific synergistic therapy *via* disruption of mitochondrial Ca^{2+} homeostasis and photothermal therapy. ACS Nano 2018;12(7):6806–18.
[2] Dong Z, Feng L, Hao Y, Li Q, Chen M, Yang Z, et al. Synthesis of $CaCO_3$-based nanomedicine for enhanced sonodynamic therapy *via* amplification of tumor oxidative stress. Chem 2020;6(6):1391–407.
[3] Zheng P, Ding B, Shi R, Jiang Z, Xu W, Li G, et al. A multichannel Ca^{2+} nanomodulator for multilevel mitochondrial destruction-mediated cancer therapy. Adv Mater 2021;33(15):2007426.
[4] Ding B, Zheng P, Jiang F, Zhao Y, Wang M, Chang M, et al. MnO_x nanospikes as nanoadjuvants and immunogenic cell death drugs with enhanced antitumor immunity and antimetastatic effect. Angew Chem Int Ed 2020;59(38):16381–4.
[5] Liu D, Chen B, Mo Y, Wang Z, Qi T, Zhang Q, et al. Redox-activated porphyrin-based liposome remote-loaded with indoleamine 2,3-dioxygenase (IDO) inhibitor for synergistic photoimmunotherapy through induction of immunogenic cell death and blockage of IDO pathway. Nano Lett 2019;19(10):6964–76.
[6] Feng XR, Xu WG, Liu JH, Li D, Li G, Ding JX, et al. Polypeptide nanoformulation-induced immunogenic cell death and remission of immunosuppression for enhanced chemoimmunotherapy. Sci Bull 2021;66(4):362–73.
[7] Sun YJ, Feng XR, Wan C, Lovell JF, Jin HL, Ding JX. Role of nanoparticle-mediated immunogenic cell death in cancer immunotherapy. Asian J Pharm Sci 2021;16(2):129–32.
[8] Zheng P, Ding B, Jiang Z, Xu W, Li G, Ding J, et al. Ultrasound-augmented mitochondrial calcium ion overload by calcium nanomodulator to induce immunogenic cell death. Nano Lett 2021;21(5):2088–93.