Precise nano-programmed ferroptosis with enhanced tumor chemotherapy

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Cancer treatment is one of the greatest challenges in biomedical research, and the development of novel tumor-inhibition mechanisms has long been a topic of interest for researchers in this field. Ferroptosis is one such candidate that holds promise for clinical translation. It is a recently discovered non-apoptotic model of regulated cell death that shows high iron-dependence. Previous reports collectively revealed that ferroptosis is fundamentally different from other forms of regulated cell death such as apoptosis, necrosis and pyroptosis in terms of the morphological, biochemical and genetic features, which may offer new opportunities for the treatment of tumor indications that are resistant to conventional antitumor modalities [1–3]. However, reports on the implementation and evaluation of ferroptosis-based tumor therapy are still rare. Very recently, Yu and Luo *et al.* [4] reported a novel strategy to initiate and enhance ferroptosis in target tumor cells using clinically tested drugs and inorganic nanocomponents (Fig. 1a).

Due to the central role of Fe^{2+} ions in the ferroptosis process, it is theorized that disrupting the iron homeostasis in tumor cells *via* Fe^{2+} delivery could be a viable strategy to trigger ferroptotic cell death for tumor inhibition [5–7]. However, Fe^{2+} ions are very sensitive to the oxidative stress in the biological environment and their pro-ferroptotic activity is further affected by the endogenous supply of reactive oxygen species (ROS). In this study, the authors discovered that Fe^{2+} ions could coordinate with doxorubicin (DOX), a topoisomerase 2-inhibitor commonly used for chemotherapy, to form a drug-metal coordination complex. The Fe^{2+} -DOX complex was highly stable under physiological conditions, but could be dissociated into Fe^{2+} and free DOX molecules under acidic pH of around 5–6. In addition to the en-

hanced oxidation resistance, the DOX could also activate the NADPH oxidase 4 (NOX4) in tumor cells to generate a large amount of H_2O_2 to supply the iron-catalyzed lipid peroxidation, eventually leading to amplified ferroptotic damage.

Based on the rationales discussed above, the authors successfully prepared amorphous calcium carbonate (ACC) nanoparticles intercalated with Fe^{2+} -DOX complex *via* a simple self-assembly-based one-step co-condensation protocol, which could be readily disintegrated into Ca^{2+} , CO_2 , DOX and Fe^{2+} in the acidic tumor intracellular environment (Fig. 1b–h) [8,9]. To enhance the tumor specificity of the nanoformulation, the ACC-based cores were further modified with sequentially-responsive polyamidoamine (PAMAM) dendrimer-based ligands. The dendrimers were functionalized with either polyethylene glycol monomethyl ether (mPEG) *via* matrix metalloproteinase-2 (MMP-2)-responsive linkers or the tumor-targeting folic acid moieties, which could improve the circulation stability of the nanoformulation while being capable of switching to a tumor-affinitive state in the MMP-2-rich tumor microenvironment. Moreover, the PAMAM dendrimer could disrupt the lysosomes after the folic acid-assisted uptake, which could facilitate the interaction of Fe^{2+} and DOX with the corresponding cytosolic targets.

In addition to the mechanistic investigation on the complementary ferroptosis/apoptosis action of the ACC-based nanoformulation, Yu and Luo *et al.* [4] further studied its antitumor efficacy against multiple types of tumor indications including 4T1 breast cancer mouse models and A375 melanoma mouse models. The *in vivo* experiments evidently demonstrated that the treatment with the ACC-based nanoformulation has significantly

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Figure 1 (a) Construction process and therapeutic mechanism of the ACC-based nanoformulation. TEOS: tetraethyl orthosilicate. (b–g) Scanning electron microscopic and transmission electron microscopic images of the ACC nanoformulation intercalated with Fe²⁺-DOX complex. (h) Scanning transmission electron microscopy image of the nanoformulation and the corresponding energy-dispersive spectroscopy (EDS) results. (i) Molecular pathway for the complementary ferroptosis/apoptosis action induced by the Fe²⁺/DOX complex. Reproduced with permission from Ref. [4], Copyright 2020, American Association for the Advancement of Science.

prolonged the survival of tumor-bearing mice in comparison with those that only received DOX medication (Fig. 1i). Meanwhile, the nanoformulation also ameliorated the DOX-induced cytotoxic effects against healthy tissues and organs, thus improving the overall reliability and safety of the antitumor treatment.

In summary, the work by Yu and Luo *et al.* [4] offers a

novel prospect for the development of ferroptosis-based therapies through the strategic combination of ferroptosis-inducing Fe²⁺ ions and DOX as well as the rationally functionalized ACC nanostructures, which could efficiently trigger ferroptosis in target tumor cells with optimal safety. This work also opens up a new avenue for repurposing old anticancer drugs for new therapeutic

mechanisms, which is beneficial for the clinical translation of the nanoformulation. It should also be noted that the ACC-based nanoformulation itself is highly modulable, which could be applied as a general treatment modality against a broad spectrum of tumor indications.

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