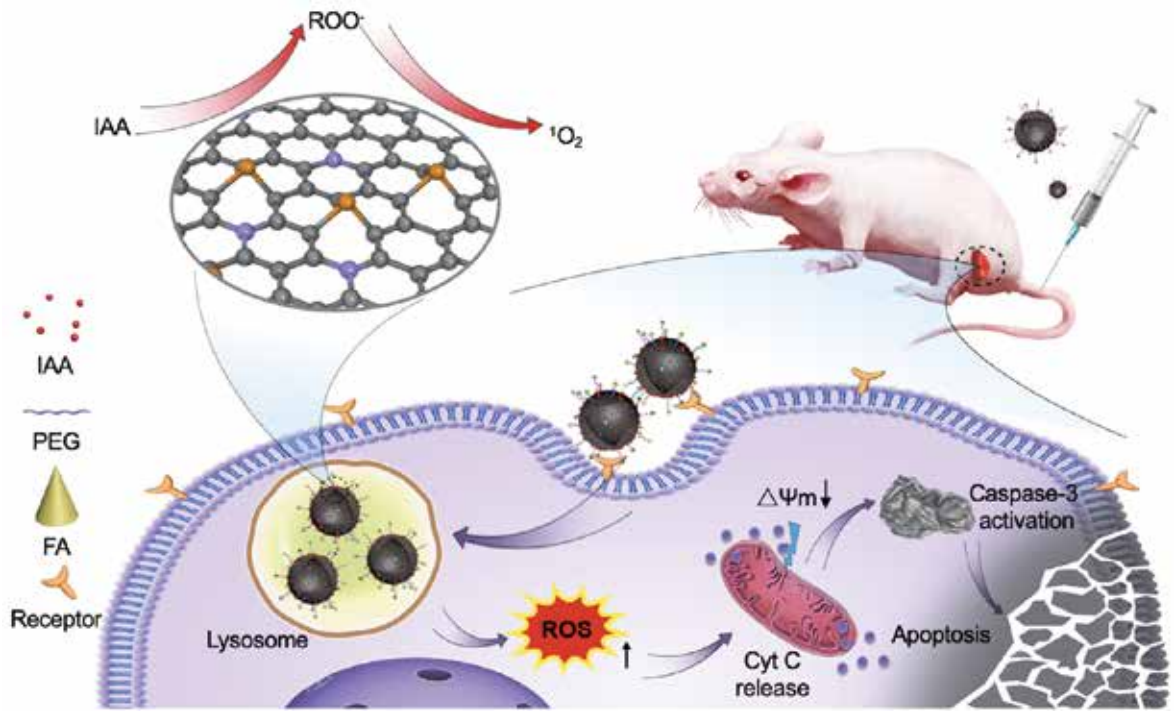
Life Sciences

A Metal-free Nanozyme-activated Prodrug Strategy for Targeted Tumor Catalytic Therapy

Enzyme prodrug therapy, which was introduced in 1985, is an attractive strategy for targeted cancer therapy with low systemic toxicity. The prodrug itself is low-toxic or non-toxic, after it was ingested, the enzyme can selectively activate the prodrug at the site of the lesion, thus enhancing the pharmacological effect and reducing the side effects.

Due to the deficiency of endogenous enzyme activity and its wide distribution in normal tissues, exogenous enzyme prodrug therapy strategy had attracted researchers' attention. Among them, horseradish peroxidase (HRP) and indole-3-acetic acid (IAA) have been demonstrated as an effective enzyme prodrug system. HRP can catalyze the prodrug IAA to produce highly toxic free radical products and kill tumors effectively, so it has a broad application prospect in tumor treatment. HRP can be selectively introduced into tumor cells through antibody conjugation or gene delivery. However, conjugated antibody targeting is limited by missed target *in vivo* and acting outside tumor cells, leading to its deficiency in tumor killing. Meanwhile, the selective expression of exogenous *HRP* in tumor cells is difficult, and transgenic safety is also a problem. Therefore, a peroxidase mimic enzyme with



Schematic illustration of the metal-free nanozyme-activated prodrug strategy for targeted tumor catalytic therapy. (Image by Dr. FAN Kelong's group)

tumor targeting and enzyme activity is needed.

Since our first report of peroxidase-like activity of Fe_3O_4 nanoparticles in 2007, nanozymes have grown into a superfamily of artificial enzymes. Due to the advantages of robust catalytic activity, high stability, simple preparation and low-cost, nanozymes have exhibited great potentials in biosensors, environmental protection and anti-biofouling. Recently, significant progresses have been made in applying nanozymes for disease diagnosis and as therapeutics *in vivo*. As many of the nanozymes are made from metal or metal oxide and susceptible to destabilizing oxidation under acidic conditions, their *in vivo* applications are limited and often complicated by the potential metal toxicity and long-term biosafety. Thus, the metal-free nanozymes that possess robust activities are desirable to achieve effective *in vivo* theranostic applications.

Herein, under the guidance of theoretical predictions, we designed and fabricated a novel phosphorous (P) and nitrogen (N) dual-doped porous hollow carbon sphere nanozyme (PNCNzyme) that exhibits a significantly improved peroxidase-like activity as compared to single N doped counterparts. A prodrug activation strategy for tumor catalytic therapy was then developed based on the as-prepared metal-free nanozyme.

It has been shown that IAA could be effectively loaded onto PNCNzymes to form PNCNzymes@IAA nanoparticle via π-π interactions, which does not affect the enzymatic activity of PNCNzymes. Moreover, the modification of folate (FA) ensures the tumor targeting and effective endocytosis of PNCNzymes@IAA nanoparticles in tumor cells. Once located in lysosomes under acidic conditions, the peroxidase activity of PNCNzyme effectively activate the prodrug IAA to produce abundant free radical intermediates, which subsequently trigger reactive oxygen species (ROS) storm and ultimately leads to apoptosis of cells through the mitochondrial pathway, as illustrated in the figure.

In addition, the formulations of PNCNzyme-IAAs system exhibited excellent biosafety *in vivo*. Thus, the strategy achieves delivering prodrugs, targeting tumor cells and activation of prodrugs in one nanoparticle. Taken together, this rationally designed nanozyme based-strategy achieves efficient activation of prodrug selectively in tumor sites, and may inspire new approaches for tumor catalytic therapy.

Contact:
FAN Kelong
Institute of Biophysics, Chinese Academy of Sciences
Beijing 100101, China
Email: fankelong@ibp.ac.cn

Reference

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