CANCER

Apoptotic body–mediated intercellular delivery for enhanced drug penetration and whole tumor destruction

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Chemotherapeutic nanomedicines can exploit the neighboring effect to increase tumor penetration. However, the neighboring effect is limited, likely by the consumption of chemotherapeutic agents and resistance of internal hypoxic tumor cells. Here, we first propose and demonstrate that apoptotic bodies (ApoBDs) could carry the remaining drugs to neighboring tumor cells after apoptosis. To enhance the ApoBD-based neighboring effect, we fabricated disulfide-linked prodrug nanoparticles consisting of camptothecin (CPT) and hypoxia-activated prodrug PR104A. CPT kills external normoxic tumor cells to produce ApoBDs, while PR104A remains inactive. The remaining drugs could be effectively delivered into internal tumor cells via ApoBDs. Although CPT exhibits low toxicity to internal hypoxic tumor cells, PR104A could be activated to exert strong cytotoxicity, which further facilitates deep penetration of the remaining drugs. Such a synergic approach could overcome the limitations of the neighboring effect to penetrate deep into solid tumors for whole tumor destruction.

INTRODUCTION

Chemotherapeutic nanomedicines have been found to enhance drug penetration in solid tumors and amplify their therapeutic effects through the neighboring effect. The neighboring effect means that tumor cells may become in situ drug depots after taking up drug-loaded nanoparticles (NPs). When drug-loaded NPs induce cell apoptosis, the remaining drugs are liberated from dead or dying cells, which then infect surrounding cells (*1*–*3*). The detailed mechanism behind the transport of the drugs from the depot cells to other cells needs to be further elucidated. Yong and coworkers (*4*) reported that the neighboring effect of NPs might be modulated via lysosomal exocytosis. Zhou *et al.* (*5*) proposed that the cationization of NPs could effectively induce adsorption-mediated transcytosis, which was favorable for tumor penetration. However, we suppose that there may be other routes for the effective intercellular transport of large amounts of NPs after tumor cell death.

When tumor cells undergo apoptosis, the cell membrane shrinks, divides, and wraps the cytoplasm to produce apoptotic bodies (ApoBDs) (*6*). Therefore, the remaining drugs may be stored in ApoBDs. The elimination of apoptotic cells is mainly accomplished by "professional phagocytes" such as macrophages or "nonprofessional neighboring cells." Professional phagocytes are often not abundant in the sites where apoptosis occurs. Therefore, nonprofessional neighbors usually clear apoptotic cells during development (*7*–*9*). In addition, given the nutrient-deprived conditions of tumor cells to proliferate, they generally adsorb nutrients through macropinocytosis with the scavenging of macromolecules from the microenvironment, such as ApoBDs (*10*, *11*). Hence, we propose that the macropinocytosis of ApoBDs by neighboring tumor cells may contribute to the efficient intercellular drug delivery of the neighboring effect.

Theoretically, the neighboring effect could facilitate chemotherapeutic agents to continuously penetrate deep into the tumor until it kills all tumor cells like "peeling an onion" layer by layer. However, according to previous reports, the neighboring effect is an effect of limited tumor penetration distance (*2*). The limitations of penetration may be ascribed to the consumption of chemotherapeutic agents during the peeling an onion process and the resistance of internal hypoxic tumor cells (*12*, *13*). Hypoxia-activated prodrugs (HAPs) are a class of drugs that maintain nontoxicity under normoxia but can be converted into toxic drugs by highly expressed reductases in the hypoxic tumor region (*14*–*16*). Therefore, combining a chemotherapeutic agent with an HAP may provide a synergic approach to overcome the limitations of the neighboring effect for a "relay race" tumor deep penetration and the entire tumor-killing effect.

Prodrug nanoassemblies are a new type of self-delivering nanoplatform by the drug conjugates, demonstrating a variety of advantages, such as facile fabrication, high drug-loading capacity, extended blood circulation, and reduced carrier-related toxicity (*17*–*20*). In addition, redox-responsive nanodrug delivery systems have attracted widespread attention due to the significantly different redox levels between tumor cells and normal cells (*21*, *22*). Therefore, self-assembled prodrug NPs with redox-sensitive drug release behavior hold great potential for application in combination treatment.

Here, we prepared CSSP NPs through the self-assembly of the heterodimeric prodrug CPT-SS-PR104A consisting of camptothecin (CPT), HAP PR104A, and a disulfide linkage (Fig. 1A). As expected, the high-level cytosolic glutathione (GSH) could trigger the cleavage of the disulfide bond to release CPT and PR104A quickly. CPT killed external normoxic tumor cells to produce CPT- and PR104A-coloaded ApoBDs. ApoBDs can be engulfed by neighboring cells to deliver drugs into internal hypoxic tumor cells. The drug content of CPT was gradually consumed by the normoxic cells, and the hypoxic cells were also resistant to CPT, while PR104A with low consumption could be activated to exert cytotoxicity in the hypoxic cells for further drug penetration (Fig. 1B). Our research indicates that the ApoBD-mediated neighboring effect could facilitate CSSP NPs to

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