Stem cells as a photosensitizer carrier to attack cancer cells for photodynamic therapy of breast cancer

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Statement of Purpose: Photodynamic therapy (PDT) combines non-toxic photosensitizer (PS), harmless visible light, and cell- and tissue-associated oxygen to generate cytotoxic reactive oxygen species (ROS) for treating cancer cells.[1] However, current PDT is limited by the difficulty in specifically delivering PS to the target site, which in turn limits the photodynamic efficiency. It is a great challenge in PDT of cancer to find a new strategy for delivering PS to cancer cells and tumors to achieve targeted destruction of tumors. Recently, it has been reported that mesenchymal stem cells (MSCs) can migrate to tumors and tend to be distributed and retain at the tumors.[2] Therefore, we employed MSC as a carrier to deliver PS to breast tumors for treatment (Fig. 1).

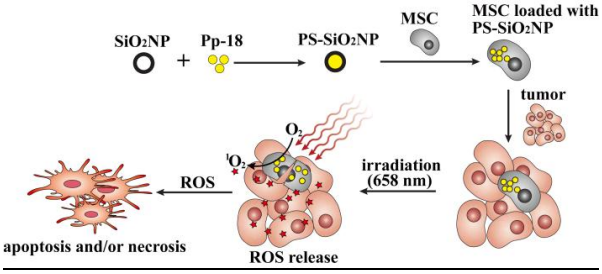

Figure 1. Schematic illustration.

Methods: We first synthesized porous silica nanoparticles (SiO_2NPs) and then loaded a PS, called purpurin-18 (pP-18), into the pores to form PS-loaded SiO_2NPs (PS-SiO_2NPs). The successful loading of PS into SiO_2NPs was verified by a weight loss of PS-SiO_2NPs after heating under a temperature of ~150-300^{o}C. Then PS-SiO_2NPs were incubated with MSCs at 37 ^{o}C for 4 hours at 37 ^{o}C and the uptake of PS-SiO_2NPs into MSCs was verified by viewing the green fluorescence of a dye-peptide-SiO_2NP (where a dye-labeled peptide was used to replace PS) from MSCs. To verify the generation of ROS inside MSCs upon the irradiation of a light (with a power density of 0.04W/cm^2) on PS-SiO_2NPs-MSCs, we used a DCFH-DA staining kit to incubate MSCs with DCFH-DA for 30 min. The ROS will be stained with the DCFH-DA dye to show green fluorescence. Thus the level of ROS inside MSCs was directly related to the intensity of green fluorescence. To test the anti-tumor effects for PS-SiO_2NPs-MSCs *in vivo*, we co-inject the MSCs and MCF-7 cancer cells on the backs of nude mice. The PDT was initiated by irradiating a red light onto the injected area. Three groups were performed in this test: Group 1 received a co-injection of MCF-7 and PS-SiO_2NPs-MSCs. The injected area was irradiated for 15 min to initiate PDT one day after injection. Group 2 was same as Group 1 except that the irradiation was applied to trigger PDT 5 days after injection. Group 3 as a control received a co-injection of MCF-7 and MSCs without loading PS-SiO_2NPs, followed by light irradiation on the injected area for 15 min one day after injection. After the treatment, the tumor size and weight were both measured and H&E staining of tumor tissues was performed to determine the anti-tumor effects of PS-SiO_2NPs-MSCs.

Results: Our TEM, SEM, and BET data showed that our synthesized mesoporous silica nanoparticles had a uniform size of ~230 nm with a large surface area of 234.88 m^2/g. The thermal gravimetric analysis suggested 31% wt organic PS was burned during heating and verified the successful loading of pP-18 into SiO_2NPs. MTT assay results indicated that in the absence of light irradiation PS-SiO_2NPs did not show significant toxicity to MSCs when their concentration was lower than 80 μg/ml. To evaluate the uptake of PS-SiO_2NPs by MSCs, we used FITC-labeled peptide-SiO_2NP as a substitute and found green fluorescence around cell nuclei, suggesting the internalization of green-dye-loaded SiO_2NPs inside MSCs. The fluorescently activated cell sorting analysis further confirmed that more than 90% MSCs were fluorescent due to the uptake of peptide-loaded SiO_2NPs. Then we performed *in vitro* migration assay[3] to check whether the MSCs loaded with PS-SiO_2NPs (PS-SiO_2NPs-MSCs) would inhibit the migration of MSCs to MCF-7 breast cancer cells. The results showed that the loading of PS-SiO_2NPs in MSCs did not significantly reduce the number of MSCs that migrated to MCF-7 cells. To verify the generation of ROS inside MSCs upon the irradiation of a light on PS-SiO_2NPs-MSCs, a DCFH-DA staining kit was used. As expected, upon light irradiation on MSCs loaded with PS-SiO_2NPs with different concentrations, intracellular ROS level was increased with the concentration of PS-SiO_2NPs used to interact with MSCs. This fact indicates that the internalization of PS-SiO_2NPs in MSCs resulted in the presence of PS in MSCs, which was activated by light to trigger the excitation of oxygen into singlet oxygen.

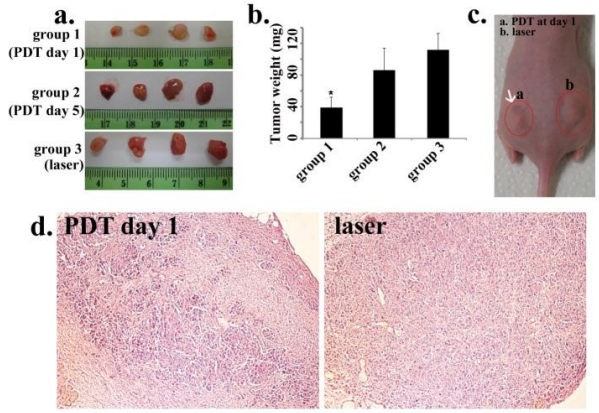

Figure 2. *In vivo* PDT of PS-SiO_2NPs-loaded MSC.

At last, we performed the *in vivo* anti-tumor test. We found that the tumor size and weight were reduced in Group 1 compared to Group 2 and 3 (Figs. 2a&b). This indicates that PDT at day 1 after injection (Group 1) generated ROS to inhibit tumor growth whereas the absence of PS-loaded MSCs (Group 3) did not induce PDT even under light irradiation. H&E staining of tumor

tissues of Groups 1 and 3 also verified the death of cells in the tumors in Group 1 (Fig. 2d). It is interesting that PDT at day 5 after injection (Group 2) did not induce additional inhibition compared to PDT at day 1, probably because MSCs co-injected with MCF-7 cells could stimulate and support tumor tissue once they were present in the tumor microenvironments before PDT was applied on Day 5. These results show that PDT needs to be initiated soon after the arrival of MSCs in tumor sites when MSCs are used as a carrier for drug delivery.

Conclusions: We took advantage of the tumor-homing capability of MSCs[2] to deliver PS to cancer cells and tumors. We successfully found that internalization of PS-loaded SiO_2NPs did not induce significant toxicity against MSCs, nor did they significantly inhibit the tumor-homing capability of MSCs. When the PS-loaded MSCs were injected into MCF-7 bearing tumors, the tumor growth was inhibited, suggesting the retention of MSCs (and thus the PS loaded in MSCs) in tumors and the consequent destruction of tumors by PDT. Since many drugs can be loaded into the biocompatible silica nanoparticles, which can be further internalized by MSCs by mechanisms such as endocytosis, the use of MSCs as a natural carrier to deliver drug to tumors is a promising approach to targeted cancer therapy.

References:

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