Multifunctional lipid nanoparticles remodeling tumor immune microenvironment for breast cancer chemo-immunotherapy

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Background

- Breast cancer remains a leading malignancy among women, with therapeutic efficacy often limited by the immunosuppressive tumor microenvironment (TME). Combining DOX with resiquimod (R848), a TLR7/8 agonist, can boost immune responses and macrophage polarization, enhancing anti-tumor activity. This study develops a chitosan-based liposomal nanodrug system co-loading DOX and R848 to target tumor cells and tumor-associated macrophages (TAMs), aiming to improve breast cancer therapy.

Methods

- Folic acid (FA) and chitosan (CS) were conjugated to form the FA-CS complex, which encapsulated liposomes to enhance stability and targeting. FA-CS-R848/DOX@Lip liposomes were prepared by rotary evaporation, and their morphology, size, and zeta potential were characterized by TEM and DLS, respectively. Drug encapsulation efficiency (EE), drug loading (LD) capacity, and release profiles were evaluated by HPLC. Cellular uptake and cytotoxicity were determined by LSCM and CCK8, respectively. In vivo, a mouse breast cancer xenograft model was used to assess anti-tumor efficacy and biosafety. The macrophage phenotype, TME composition, and CD8$^+$ T cell cytotoxic function were evaluated by flow cytometry.

Results

- The FA-CS-R848/DOX@Lip showed a spherical and uniform shape, with an average size of 211 nm and a zeta potential of 5.3 mV. (Figure 1 and Table 1)

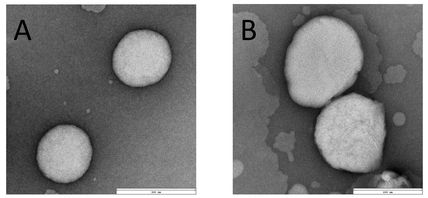


Figure 1. TEM images of R848/DOX@Lip (A) and FA-CS-R848/DOX@Lip (B).

Table 1. Malvern particle size and zeta potential of R848/DOX@Lip and FA-CS-R848/DOX@Lip (n=3).

	Size (nm)	PDI	zeta potential (mv)
R848/DOX@Lip	195.4 ± 1.49	0.17 ± 0.03	-21.8 ± 3.96
FA-CS-R848/DOX@Lip	211.0 ± 3.96	0.24 ± 0.02	5.3 ± 0.41

- The EE (%) of R848 and DOX were over 80% and 70%, respectively, while the DL (%) capacities were 6.8% and 4.6%. Drug release studies showed a slow release profile.(Table 2 and Figure 2)

Table 2. EE (%) and DL (%) of DOX and R848 in R848/DOX@Lip and FA-CS-R848/DOX@Lip liposomes (n=3).

	EE% (DOX)	EE% (R848)	DL% (DOX)	DL% (R848)
R848/DOX@Lip	86.6 ± 1.6	73.7 ± 1.9	4.5 ± 0.4	6.8 ± 0.3
FA-CS-R848/DOX@Lip	83.3 ±2.1	70.5 ± 2.8	3.1 ± 0.5	4.6 ± 0.2

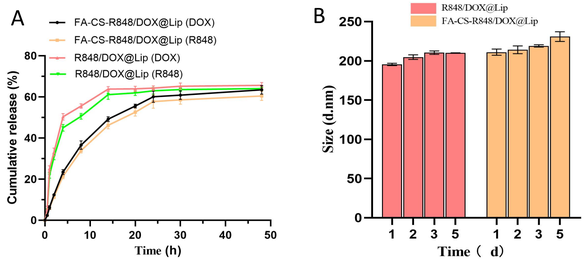


Figure 2. Cumulative release rates of DOX and R848 in liposomes (A) (n=3). In vitro stability of liposomes (B) (n=3).

- In cytotoxicity tests, FA-CS-R848/DOX@Lip demonstrated enhanced anti-tumor activity by inducing tumor cell death and stimulating immune responses. (Figure 3)

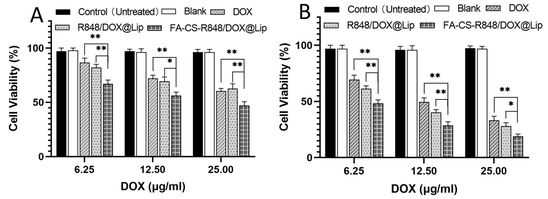


Figure 3. Cytotoxicity study of different treatment groups after 24 hours (A) and 48 hours (B) incubation with EO771 cells. n=3.

- Cellular uptake assays revealed significantly higher uptake of FA-CS-R848/DOX@Lip in EO771 tumor cells and RAW 264.7 macrophages compared to non-targeted liposomes. (Figure 4)

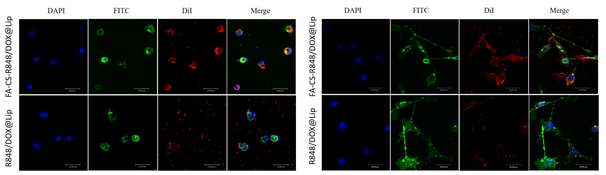


Figure 4. Targeting ability of liposomes toward EO771 tumor cells and RAW264.7 macrophages.

- In vivo, FA-CS-R848/DOX@Lip significantly suppressed tumor growth with good biocompatibility. (Figure 5 and 6)

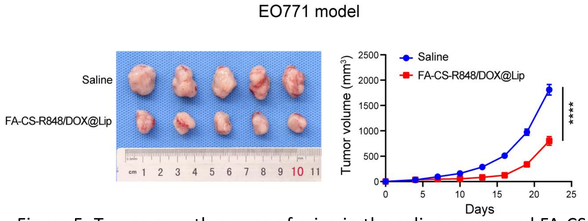


Figure 5. Tumor growth curves of mice in the saline group and FA-CS-R848/DOX@Lip group.

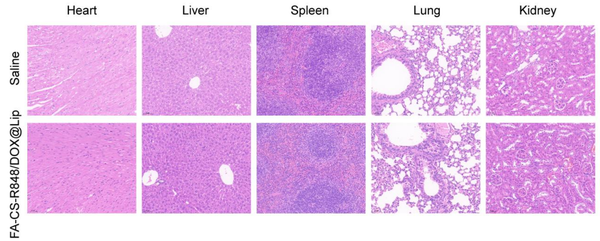


Figure 6. H&E staining of major organs from mice in the saline group and FA-CS-R848/DOX@Lip group.

- FA-CS-R848/DOX@Lip increased the MHCII/CD206 ratio of TAMs, elevated the proportions of CD8+ and CD4+ T cells, reduced the proportion of MDSCs, and enhanced the percentages of IFNγ+ and Ki67+ CD8+ T cells in vivo. (Figure 7)

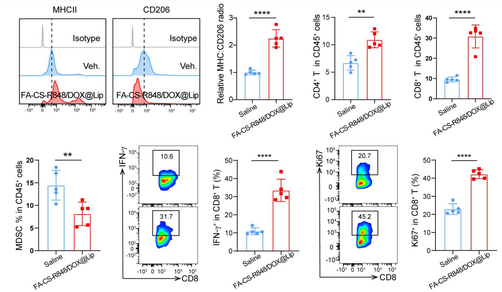


Figure 7. Flow cytometry analysis showed that FA-CS-R848/DOX@Lip group remodels the tumor immune microenvironment in mice, enhancing CD8+ T cell function.

Conclusions

Our results indicate that FA-CS-R848/DOX@Lip exhibits a potent antitumor effect and good biocompatibility in mouse models, with synergistic effects between chemotherapy and immunotherapy, potentially providing a new avenue for breast cancer treatment.

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