

Cannibalism-inspired biomimetic nanovesicles achieve boosted tumor targeting capacity via senescence-induced membrane remodeling

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Abstract

Conventional biomimetic tumor-targeting carriers are hindered by insufficient target molecule density, unstable intercellular adhesion, and complex modification requirements. Inspired by cell cannibalism phenotype of senescent tumor cells, we developed senescent tumor cell membrane nanovesicles (STCM-NVs) that utilize senescence-induced cell membrane remodeling strategy to amplify native targeting capacity. We found that senescence induction achieves dual remodeling of tumor cell membranes: molecularly, it upregulates the expression of cell adhesion molecules and exposes multiple targeting sites to enhance STCM-NVs tumor recognition; morphologically, it promotes the formation of dense filopodia to stabilize STCM-NVs-target cells adhesion and prevent shear stress-mediated off-target detachment during systemic circulation. *In vitro*, STCM-NVs showed a 3.3-fold higher targeting affinity index than non-senescent tumor cell membrane-NVs (HTCM-NVs). *In vivo*, based on tumor-to-liver fluorescence intensity ratios, STCM-NVs achieved more than 6-fold improvement in tumor-targeting capacity relative to HTCM-NVs. Moreover, STCM-NVs also exhibited superior tumor-accumulation kinetics featuring more rapid tumor uptake, an earlier accumulation peak, and prolonged intratumoral retention. After loading with a therapeutic agent, STCM-NVs significantly increased the tumor inhibition rate from 71.68% (for HTCM-NVs) to 90.74% in an orthotopic breast cancer mouse model. Further mechanistic studies revealed that Fibronectin-1 and Collagens serve as the core targeting molecule and internalization mediators, respectively, which promote efficient caveolae-dependent cellular uptake through F-actin reorganization. Overall, this study establishes a mechanistic foundation for using cellular physiological phenotypes to enhance innate biological processes and offers a translatable strategy for targeted therapies.

Highlights

- Tumor cell membrane targeting is dynamically modulated by cellular phenotype, instead of a static property.
- Senescence-induced membrane remodeling upregulates adhesion molecules and induces filopodia formation.
- Senescent tumor cell membrane nanovesicles retain remodeling phenotypes with superior tumor targeting, enhanced cell adhesion, and efficient internalization.
- Fibronectin-1, Collagen, and F-actin cooperate to mediate tumor recognition, internalization, and trafficking for effective drug delivery.

1. Background

Low tumor enrichment remains a serious and urgent challenge for drug delivery in cancer therapy [1–2]. Cancer cell membrane-based (CCM-based) biomimetic camouflaged nanocarriers have attracted consideration attention because of their multiple advantages, including the lower immunogenicity, homologous targeting capacity, a prolonged circulation time, and ability to traverse biological barriers,

features that are difficult to achieve with artificial materials [3–5]. However, the insufficient density of homologous ligands in single-type CCM along with cancer heterogeneity, hinders their ability to achieve substantial tumor targeting [6–7]. Therefore, the fabrication of CCM-based carriers modified with targeting ligands or peptides to improve drug delivery efficiency and therapeutic effects at the desired site has become an attractive strategy [8–9].

Currently, researchers primarily use physical, chemical, and genetic modification as well as membrane hybridization, to functionalize CCM-based carrier [9–12]. Despite some progress in enhancing tumor targeting, the existing approaches still suffer from considerable limitations in the following aspects: (i) The membrane surface modifications are susceptible to inactivation under physiological conditions or within the tumor microenvironment [13]. (ii) Because cancer cell membranes present multiple composite targeting molecules, thus the single-target modification strategies are more prone to off-target effects during tumor progression and are more susceptible to immune recognition and clearance after repeated administrations [14]. (iii) It is difficult to precisely controlling the extent of cell membrane modification, overly dense ligand packing can cause non-cooperative effect on target receptor binding, increased uptake by immune cells, and nonspecific binding to perivascular cells after extravasation [15]. (iv) Surface biochemical conjugation used to introduce targeting properties to CCM may disrupt membrane structure and function and compromise membrane protein activity [16]. (v) many physicochemical methods are overly complicated or unsuitable for clinical translation [17–19]. Therefore, exploiting the appropriate native cell membrane with inherent biological functions and tumor targeting properties along with efficient fabrication strategies, rather than mainly relying on artificial biochemical conjugation to enhance targeting effect, warrants further investigation.

Recent studies have revealed a previously unrecognized ability of chemotherapy-induced senescent cancer cells to engulf neighboring healthy cancer cells at a remarkable frequency [20–22]. This cannibalism phenotype has been evidenced in chemotherapy-induced senescent tumors and cell lines, but did not exist in normal healthy cancer cells [21]. A related study showed that the senescent cancer cells, acting as “predators”, actively and precisely localized to the membrane edge of “prey” cells (homotypic healthy cancer cell) [20]. The advancing filopodial structures of senescent cancer cells consistently attach to the neighboring healthy cancer cells membranes [20]. These findings suggested that senescent cancer cells acquire a phagocytic phenotype that enables efficient targeting of homotypic healthy cancer cells. Although the mechanisms underlying this engulfment process remain incompletely understood [23–24], this efficient targeting and adhesion capability conferred by senescent cancer cells could potentially be harnessed for therapeutic application.

Based on this concept, we designed a senescent tumor cell membrane based nanovesicles (STCM-NVs) delivery system and evaluated its targeting adhesion, internalization efficiency and anti-tumor effect both *in vitro* and *in vivo*. Unlike previous studies, we conceptualized healthy cancer cells as the “predator” and the STCM-NVs as the “prey”. Notably, STCM-NVs displayed more stable interactions with cancer cells and initiated the uptake process at earlier time points compared with healthy tumor cell membrane-based nanovesicles (HTCM-NVs). Furthermore, we investigated the predominant endocytic pathway

involved in STCM-NVs mediated internalization and identified the cell adhesion molecule fibronectin-1 (FN1) as a key mediator of selective tumor cell targeting mediated by STCM-NVs. In addition, we unveiled that alterations in collagen structure and F-actin remodeling were directly correlated with the cellular uptake efficiency of STCM-NVs. All together, these findings support the potential application of STCM-NVs as a novel drug-delivery platform with efficient tumor targeting capability (Scheme 1).

2. Results

2.1 Generation and Characterization of STCM-NVs

In this study, the breast cancer cell line MDA-MB-231 cells were selected as the supplier of membrane, given that they have been reported to exhibit a cannibalistic phenotype [21]. To prepare STCM-NVs, the proliferating breast cancer cells were treated with increasing concentration of doxorubicin (DOX) for 6 ~ 7 days. Initially, cellular senescence was monitored by assessing senescence-associated β -galactosidase (SA- β -gal) activity, SA- β -gal staining results showed that DOX at a concentration higher than 0.15 μ M successfully induced cellular senescence in breast cancer cells (Fig. 1A). 5-Ethynyl-2'-deoxyuridine (EdU) incorporation assay was performed to determine the ratio of proliferation cells over induced senescent cells (Fig. 1B). As shown in S1, approximately 90% of breast cancer cells underwent cellular senescence in response to DOX induction. Meanwhile, the mRNA expression level of several major components of SASPs, including MMP9, MMP1, IL-6, CXCL8, IL-1B, and IL-1A were significantly increased both in MDA-MB-231 cells and MCF-7 cells following treatment with different concentration of DOX (Fig. 1C). Since the DOX concentration of 0.15 μ M resulted in highest cell viability, we then chose this concentration as the optimal induction conditions in the subsequent experiments. Consistent with increased mRNA expression, treatment with 0.15 μ M of DOX also remarkably increased the protein expression level of IL-6, MMP9, and IL-1 β in MDA-MB-231 cell treated (**Figure 1D**). As expected, the protein expression level of p21 and p53, cellular senescent marker was increased in breast cancer cells after treated with 0.15 μ M DOX (Fig. 1E). Taken together, these data demonstrated that a robust number of senescent tumor cells were introduced *in vitro*, which were applicable for massive membrane collection.

Subsequently, as shown in Fig. 1F, the cell membranes of senescent MDA-MB-231 cells and MCF-7 cells which prelabeled with Dil fluorescent probes were extracted and extruded through a porous polycarbonate membrane several times to generate STCM-NVs. DOX was selected as the model drug and encapsulated into the STCM-NVs (STCM-NVs@DOX) and HTCM-NVs (HTCM-NVs@DOX). Transmission electron microscopy (TEM) analysis showed that the STCM-NVs, STCM-NVs@DOX and HTCM-NVs possessed similar sizes and structures (Fig. 1G). As shown in TEM images, all these NVs exhibited typical cup-shaped membrane structure with a diameter ranging from 100 to 150 nm (Fig. 1G). To determine whether membrane proteins extracted from senescent MDA-MB-231 cancer cell were retained on the NVs, different types of membrane samples were subjected to SDS-PAGE analysis. The coomassie blue staining revealed that the STCM-NVs and HTCM-NVs preserved most of the protein components from their parental cancer cells (Fig. 1H). Nanoparticle tracking analysis (NTA) indicated that the size distribution of STCM-NVs and HTCM-NVs ranged from 100 to 300 nm (Fig. 1I), while they

did not change significantly after store at 4°C in phosphate-buffered saline (PBS) up to 80 h (Fig. 1J). Additionally, our results showed that STCM-NVs@DOX and HTCM-NVs@DOX had a similar size distribution to unloaded NVs, (Fig. 1I), indicating that DOX loading has no obvious influence on size distribution. All kinds of cell membrane based NVs (STCM-NVs, STCM-NVs@DOX, HTCM-NVs and HTCM-NVs@DOX) were negatively charged, suggesting their well solubility and stability (Fig. 1K).

2.2 The enhanced tumor targeting adhesion force and high uptake efficiency of STCM-NVs *in vitro*

To determine the degree of homotypic targeting by STCM-NVs, we treated MDA-MB-231 cells with STCM-NVs derived senescent MDA-MB-231 cells membrane prelabelled with Dil for 4 h under static culture conditions, while cells treated with HTCM-NVs served as controls. Obviously, stronger red fluorescence signals displayed at membrane edges and the inside of MDA-MB-231 cells pretreated with STCM-NVs than those pretreated with HTCM-NVs (Fig. 2A). This result suggested that the STCM-NVs possessed superior ability to form homotypic aggregates compared with HTCM-NVs. Then, subsequent experiments focus on exploring the property and function of the STCM-NVs derived senescent MDA-MB-231 cells membrane. After extending the co-incubation time from 4 h to 6 h, the confocal orthogonal view and dynamic three-dimensional reconstructions showed that the number of STCM-NVs internalized into MDA-MB-231 was much more than those of HTCM-NVs (Fig. 2A at the far right, Additional file 1–2).

Meanwhile, flow cytometry analysis further confirmed a higher fluorescence intensity of internalized STCM-NVs in MDA-MB-231 cells than those treated with HTCM-NVs (Fig. 2B), suggesting that STCM-NVs initiated the uptake process in MDA-MB-231 at an earlier time point compared to HTCM-NVs. In contrast, only faint fluorescence signals were observed in heterotypic MCF-7 cells following exposure to either STCM-NVs or HTCM-NVs (Fig. 2C, D), indicating that STCM-NVs possessed exclusive self-targeting capability. Given that the biological system is not a static environment *in vivo*, the cell membrane NVs, once entering an *in vivo* environment, therefore, will inevitably be affected by many dynamically changing factors, such as the flow shear stress (FSS) from blood flow [25]. Thus, we introduced a constant oscillating fluid flow onto MDA-MB-231 cells surface to mimic internal bloodstream shear stress (Fig. 2E Top). MDA-MB-231 cells were initially incubated with STCM-NVs or HTCM-NVs for 2 h, followed by washing thrice with PBS to remove unbound cell membrane NVs. Following fixation with 4% paraformaldehyde, and then rinsed with PBS, all dead cells maintained their superstructure while only the bound cell membrane NVs were isolated without any cellular uptake. Afterward, FSS stimulation was introduced to the cell surface for 30 min. After FSS treatment (FSS+), only a few red dots were left in HTCM-NVs treated cells (Fig. 2E Bottom). On the contrary, the fluorescence intensity of STCM-NVs in FSS+ group was almost comparable to the “NO-FSS” treated (FSS-) control group (Fig. 2E). Furthermore, STCM-NVs prepared from H₂O₂-induced senescent tumor cells also exhibited significant targeting binding to homotypic cancer cells (Fig. 2F-G), these results convincingly validating that compared to HTCM-NVs, the STCM-NVs possessed a much stronger adhesion capability toward targeted cells. Given that macrophages are specialized in phagocytosis [26],

we investigated their ability to uptake STCM-NVs. Human monocyte leukemia-derived THP-1 cells were differentiated into macrophages by treatment with 200 nM PMA for 48 h [27]. Thereafter, the cells further incubated with STCM-NVs or HTCM-NVs for 24 h. Obviously, both STCM-NVs and HTCM-NVs could evade the uptake by THP-1 macrophages, as determined by confocal microscopy and flow cytometry (Fig. 2H, I)

2.3 Evaluation of the properties of STCM-NVs as a drug carrier and the anti-tumor efficiency of STCM-NVs@DOX in vitro

To explore the biocompatibility of STCM-NVs, we monitored the blood compatibility of prepared STCM-NVs. According to the results, almost no hemolysis occurred in any group following treatment with different types of cell membrane NVs (Fig. 3A). The cytotoxicity test showed no noticeable cytotoxic effect on normal mammary epithelial cells MCF-10A, even at relatively high concentration (Fig. 3B), indicating that the low cytotoxicity of STCM-NVs carrier. We then compared the effect of different types of cells membrane NVs on cancer cell viability and found that STCM-NVs showed no obvious effect on cancer cell proliferation (Fig. 3C). This result substantiated the STCM-NVs did not have carcinogenic or cancer promoting activity. To check for real cancer cell killing efficacy, DOX was loaded in to STCM-NVs as a model and evaluated by the UV-vis spectra. As expected, the absorption peak of DOX was observed in the STCM-NVs@DOX and HTCM-NVs@DOX spectra, indicating successful loading of DOX (Fig. 3D). The maximum drug entrapment efficiency (EE%) for STCM-NVs@DOX and HTCM-NVs@DOX were calculated to be 87.37% and 87.98%, respectively (Fig. 3E) [28]. The similar concentration of DOX incorporation also leads us to deduce that any difference in overall cytotoxic effect is solely related to the initial targeting, uptake, and release of DOX from two types of carriers but not the differences in the preparation process.

Actually, we found the DOX release from STCM-NVs@DOX and HTCM-NVs@DOX displayed distinct patterns in an acellular system. As shown in Fig. 3F. Initially, the DOX release rate from STCM-NVs@DOX in the earlier phase was slower compared to the control group, but this trend was reversed in the later stages. This shift was advantageous as it might led to a reduction in the concentration of free drug in the bloodstream and then increased its accumulation in tumor. Since the STCM-NVs@DOX supposed to be intravenously administered, its pharmacokinetics *in vivo* was evaluated. Blood sample were taken at different time points after drug administration into animals. As shown in Fig. 3G, the STCM-NVs@DOX group showed a slower exponential decay and a longer blood retention time as compared with the control group with administration of free DOX. The cancer cell killing efficiency of STCM-NVs@DOX *in vitro* was first detected by CCK-8 assay. As shown in Fig. 3H, the STCM-NVs@DOX exhibited much stronger cytotoxic effect on homotypic MDA-MB-231 cells than free DOX and HTCM-NVs@DOX following treatment for 24 h, and such enhanced cytotoxic effect conferred by STCM-NVs@DOX was further accentuated with a prolongation treatment, e.g. 48 h and 72 h (Fig. 3H). Meanwhile, there was a significant increase in the percentage of apoptotic cells in STCM-NVs@DOX treated group (74.4%) compared to the HTCM-NVs@DOX treated group (57.8%) as determined by flow cytometry (Fig. 3I). The

colony formation assay further confirmed that STCM-NVs@DOX had the strongest inhibitory effect on cell proliferation and colony formation of MDA-MB-231 cells compared with other NVs (Fig. 3J).

2.4 The tumor targeting of STCM-NVs and anti-tumor effect of STCM-NVs@DOX *in vivo*

The *in vivo* experiments were performed to determine whether STCM-NVs@DOX possess enhanced tumor-targeting and drug delivery efficiency. To this purpose, 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine,4-Chlorobenzenesulfonate Salt (DID), which is frequently used for cell membrane labeling and *in vivo* NIR bioimaging [29], was embedded into the lipid bilayer of STCM-NVs and HTCM-NVs for bioimaging. Then, DID prelabeled STCM-NVs and HTCM-NVs were intravenously administered into orthotopic MDA-MB-231 tumor-bearing BALB/c-nude mice, and the NIR fluorescence signals was recorded over time (Fig. 4A). After a short period of 2 h following intravenous (i.v.) injection of STCM-NVs, Bright fluorescence signals were captured at the tumor site, but no fluorescence signals were detected at 2 h following i.v. injection of HTCM-NVs, only with weak signals observed even up to 7 h. STCM-NVs signals reached the maximum at 24 h and remained a gradually increase until 48 h post-injection (Fig. 4A). The same results were observed across multiple mice in each group (**Fig S2**). By comparison, the HTCM-NVs had a much shorter retaining time at the tumor site. Even at its maximum at 48 h post injection, the fluorescence signals of HTCM-NVs were weak in comparison to what STCM-NVs had already achieved in just 7 h post-injection and could still maintain a high level of tumor targeted concentration after 72 h post-injection (Fig. 4A). These results prospected that STCM-NVs can efficiently, rapidly, and accurately target tumor sites through the systemic circulation and maintain long-term stable accumulation. The animals were sacrificed 24 h and 48 h post-injection, and the tumor and major organs were harvested for fluorescence analysis. *Ex vivo* fluorescence analysis consistently showed that HTCM-NVs mainly distributed in organs such as liver, spleen, lung and kidney, with a minimum presence in the tumor site (Fig. 4B). In contrast, the fluorescence intensity in STCM-NVs group was mainly concentrated in the tumor site, but partially in the liver (Fig. 4B). Specifically, The tumor-to-liver fluorescence intensity ratio of STCM-NVs was more than 6-fold higher than that of HTCM-NVs (Fig. 4C).

We then compared the therapeutic efficacy of STCM-NVs@DOX, HTCM-NVs@DOX and free DOX in treating the orthotopic breast cancer model in nude mice over period of 50 days. Seven doses were administered on day 1, 8, 15, 22, 29, 36 and 43, respectively (Fig. 4D). As expected, the STCM-NVs@DOX treatment for 50 days achieved the greatest tumor inhibition compared with other treatment groups (Fig. 4E). The tumor growth curve showed that the inhibition of tumor progression achieved by STCM-NVs@DOX treatment was much more pronounced than that by treatment with either HTCM-NVs@DOX or the free DOX (Fig. 4F). At the endpoint, the tumor inhibition rate, calculated from tumor volumes, reached 90.74%, markedly higher than the 71.68% observed for HTCM-NVs control group (Fig. 4G). No unhealthy loss in weight was detectable for all the treated mice (Fig. 4H). The enhanced anti-tumor effect of STCM-NVs@DOX was further confirmed by Hematoxylin and Eosin (H&E) staining and immunohistochemistry (IHC) staining for Ki67, the marker for cell proliferation [30]. As shown in Fig. 4I,

tumor from STCM-NVs@DOX treated mice harbored larger scale of necrosis and apoptosis cells compared to those from mice treated with either HTC-M-NVs@DOX or free DOX. Meanwhile, a massive fibrous tissue (black arrow indicated) was found in STCM-NVs@DOX treated tumor tissue, which was rarely observed in other groups (Fig. 4I). The enlargement of fibrosis area often indicates tumor regression, representing significant relief in clinical cases [31]. In addition, IHC showed a significantly lower expression level of Ki67 tumors than that from other groups, further supporting the enhanced anti-tumor effect of STCM-NVs@DOX (Fig. 4J and Fig S3A).

2.5 Mechanisms underlying the enhanced tumor targeting and internalization of STCM-NVs

To investigate the molecular mechanism underlying the homotypic targeting and internalization of STCM-NVs, we first characterized surface structural alterations of MDA-MB-231 cell membrane before and after cellular senescence through scanning electron microscopy (SEM). It shown that the adjacent senescent tumor cells are connected by an abundance of filament shape pseudopodia, while shorter pseudopodia sparsely populate the membrane surface of healthy tumor cells (Fig. 5A). This result suggests the filamentous pseudopodia may facilitate the stable binding of senescent tumor cell membranes to the surfaces of homotypic cancer cells. Subsequently, the selective pharmaceutical inhibitors for three major endocytic pathways were applied to determine the principal pathway responsible for STCM-NVs mediated internalization. Pretreatment of MDA-MB-231 cells with chlorpromazine (CPZ) or 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) which targeted the clathrin-dependent endocytosis or micropinocytosis [33], resulted in slight reduction of the fluorescence spots of intracellular STCM-NVs (Fig. 5B). However, the treatment with methyl- β -cyclodextrin ($m\beta$ CD) almost completely blocked the intracellular accumulation of STCM-NVs (Fig. 5B). As reported $m\beta$ CD mainly functions to disrupt the lipid raft on the cell membrane by cholesterol depletion and blocks caveolae-mediated endocytosis[34]. This result implied that the Lipid raft was important for cellular uptake of STCM-NVs.

Lipid raft are protein-receptor-rich domains on a cell membrane and are responsible for many specific intercellular recognitions [35]. Accordingly, we hypothesize that STCMs surface may have specific targeting molecules that could be recognized efficiently by protein-receptor on lipid raft of homotypic healthy cancer cells. To test this hypothesis, we performed liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) on cell membrane proteins extracted from senescent and normal healthy MDA-MB-231 cells. We found that the protein profiles in STCM_s and HTC-Ms were remarkably different, with about 1510 differentially expressed proteins identified, among which 744 proteins were upregulated and 766 proteins were downregulated in STCM_s compared to HTC-Ms (Fig. 5C). Functionally, a consideration of the upregulated proteins was related to ECM-receptor interaction and cell adhesion molecules (Fig. 5D, red rectangle indicated). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis further revealed that the upregulation of FN1 and collagen in STCM_s was enriched in the ECM-receptor interaction pathway (Fig. 5E). It is important to note that FN1 contains RGD motif and collagen

possesses GFOGER motif [36–37]. RGD peptides and GFOGER motif are known for their strong affinity to integrin receptors, which are overexpressed on the surface of various tumor cells [37–38]. The binding interaction of RGD or GFOGER motif with integrin receptors facilitates receptor-mediated endocytosis [37, 39]. Herein, we suppose that the unique FN1 derived RGD and collagen derived GFOGER motif offer the high specificity for tumor targeting by STCM-NVs. According to previous studies, the cell adhesion molecular E-cadherin protein (CDH1) with RGD motif was proposed mainly to be mainly responsible for homotypic cancer cell targeting and mechanical connection between cells [39].

Therefore, we sought to test whether CDH1, FN1 and collagen were directly implicated in STCM-NVs tumor targeting and internalization. To this purpose, we first detected the protein expression level of collagen type I,II ,III (Col-1, Col-2 and Col-3), FN1 and CDH1 in STCM_S and HTCMT_S. As shown in Fig. 5F, the level of collagens had no obvious differences between STCMs and HTCMTs group. But the FN1 and CDH1 expression levels were much higher on STCM_S than those on HTCMT_S, consistent with the above proteomic analysis results. To further confirm whether the increased expression of FN1 and CDH1 on STCM_S were responsible for homotypic tumor targeting, we stably knocked down FN1 or CDH1 expression in MDA-MB-231 cells using two specific shRNAs targeting FN1 or CDH1 coding region. The knockdown efficiency was determined by western blot analysis (Fig. 5G and **Fig S4**). Afterwards, the STCM-NVs were prepared from shFN1 (STCM-NVs^{shFN1}) and shCDH1 (STCM-NVs^{shCDH1}) senescent MDA-MB-231 cells, respectively, followed by evaluating their membrane binding affinity and uptake efficiency by MDA-MB-231 cells. To this end, the MDA-MB-231 cells were pretreated with STCM-NVs^{shFN1} or STCM-NVs^{shCDH1} for 2 h and then subjected cells to FSS loading. Our results showed that FN1 knock down significantly reduce the binding of STCM-NVs to MDA-MB-231 cells surface, but the binding could not be blocked by CDH1 knock down (Fig. 5H and **Fig S5**). Of note, a similar difference in membrane binding was observed between HTCMT-NVs and HTCMT-NVs^{shFN1} (**Fig S6**). These findings suggested that STCM-NVs and HTCMT-NVs share similar mechanism for homologous targeting adhesion. Additionally, a prolonged incubation of STCM-NVs^{shFN1} or STCM-NVs^{shCDH1} with MDA-MB-231 cell for 6 h, the number of internalized STCM-NVs^{shFN1} and STCM-NVs^{shCDH1} showed no significant differences compared to the control group of STCM-NVs (**Fig S7**), suggesting that both STCM-NVs^{shFN1} and STCM-NVs^{shCDH1} had no effect on uptake efficiency, thereby confirming FN1 function as “recognize me” signal.

Accumulating evidences has shown that the topological structure of collagen is closely linked to its function [40–41]. To verify whether the topological structure of collagen might affect STCM-NVs targeting adhesion and uptake by homotypic tumor cells, we used multiple collagenases to pretreat MDA-MB-231 cells, followed by inducing senescence and preparing STCM-NVs^{collagen⁻}. As expected, the expression of collagen could not be detected by western blot (Fig. 5I), indicating that collagens were degraded and its structure were disrupted after collagenase treatment. Apparently, following incubation of the STCM-NVs^{collagen⁻} with MDA-MB-231 cells for 4–6 h, no internalization of STCM-NVs^{collagen⁻} was observed by target cancer cells (MDA-MB-231) (Fig. 5J). Nevertheless, the FSS stimulation had no effect on STCM-NVs^{collagen⁻} binding to the target cancer cells (**Fig S8**). Collagen appears to serve as the “eat

me” signal in this scenario. The lower targeting and accumulation of STCM-NVs^{collagen⁻} and STCM-NVs^{shFN1} at tumor site was further verified by *in vivo* animal experiment. The results showed that the fluorescence intensity at the tumor site in STCM-NVs^{collagen⁻} group and STCM-NVs^{shFN1} group were much lower than that in STCM-NVs group (Fig. 4A and Fig. 5K). The biodistribution images of STCM-NVs^{collagen⁻} and STCM-NVs^{shFN1} visually validated that the majority NVs were retained in liver tissue rather than tumor tissue (Fig. 5K). Another interesting phenomena we found that over a time-course of 1–2 h covering the early stage of internalization, confocal microscope revealed F-actin concentrated in the targeted cells at the points of contact with STCM-NVs (Fig. 5L). Following apparent internalization (4–6 hours), the concentrated F-actin disappeared and reformed uniform distribution across the cell membrane (Fig. 5L). Upon F-actin depolymerization, STCM-NVs merely bound to the target cancer cell surface and failed to undergo internalization (**Fig S9**). While in control group, the level of F-actin showed no significant difference between the contact and non-contact regions of target cells and HTCM-NVs (**Fig S10**). This result lead us propose that F-actin might enlarge caveolae structure size to promote STCM-NVs transport

Collectively, these results demonstrated that the FN1 molecule endowed STCM-NVs with the ability to identify and adhere to homologous tumor cells, and the collagen and F-actin significantly contribute to its enhanced internalization (Fig. 5M).

2.6 Effect of enhanced cannibalism frequency on the homotypic targeting efficiency of STCM-NVs

As reported senescent MCF-7 cell exhibited a higher frequency of cannibalism than senescent MDA-MB-231[21]. We stained senescent MCF-7 cells and MDA-MB-231 cells with SA-βGAL and imaged in color bright field. We found the number of cell-in-cell structure in senescent MCF-7 cells was much higher than that in senescent MDA-MB-231 cells group (Fig. 6A). Proteomic analysis indicated that senescence both upregulated cell adhesion molecules and phagocytosis related proteins (Fig. 5D and Fig. 6B). To further determine whether enhanced engulfment ability was accompanied by improved membrane targeting recognition, we compared the targeting efficiencies of NVs derived from senescent MCF-7 and MDA-MB-231 cell membrane. Firstly, DOX-induced senescence of MCF-7 cells was confirmed by assessing the levels of the senescence marker protein and major components of SASPs through qPCR and western blot (Fig. 6C-D). We then prepared STCM-NVs(MCF-7), and evaluated them by TEM imaging, and assessed their size distribution by NTA. As shown in Fig. 6E-G, the STCM-NVs derived from senescent MCF-7 cells were similar in morphology and diameter to those form senescent MDA-MB-231 cells (Fig. 1G and 1I). For targeting assay, MCF-7 cells and MDA-MB-231 cells were incubated with their corresponding labeled STCM-NVs or HTCM-NVs at equal amounts for 4 h. The confocal microscopy analysis suggested that STCM-NVs(MCF-7) exhibited a significant enhancement in targeting binding over their non-senescent counterparts. However, this enhancement was not significantly greater than that observed for STCM-NVs derived from senescent MDA-MB-231 cells (Fig. 6H). We extended the analysis of membrane characteristics of senescent MCF-7 cells. SEM results revealed that senescent

MCF-7 cells also displayed dense filopodial structures on the membrane surface, but the density was not markedly increased compared with that of senescent MDA-MB-231 cells (Fig. 6I). At the molecular level, the expression of FN1, a core mediator of targeting recognition and adhesion, was upregulated in senescent versus non-senescent MCF-7 cells, yet showed no significant difference relative to senescent MDA-MB-231 cells. In contrast, p53 expression, which is directly associated with cannibalism frequency[21], was significantly higher in senescent MCF-7 cells than in senescent MDA-MB-231 cells (Fig. 6J). Together, these findings demonstrate that enhanced engulfment and improved tumor-targeting are decoupled in senescent tumor cells. This notion rationalizes the comparable targeting enhancement observed for senescent membrane vesicles derived from two cell lines that differ in their cannibalism frequency.

2.7 The biosafety of STCM-NVs@DOX *in vivo*

As for any experimental nanocarriers for drug delivery, there is always a cause for concern about the potential *in vivo* toxicity. To evaluate the biosafety of STCM-NVs@DOX *in vivo*, a panel of serum-related parameters were tested. As shown in Fig. 7A-C, the values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA) and albumin/globulin ratio (A/G) were all within the normal range, indicating that STCM-NVs@DOX did not cause any adverse effect on the murine liver and kidney. Generally, an elevated levels of IL-4 and IL-10 is associated with immunosuppression [32]. ELISA results showed that the serum levels of IL-4 and IL-10 were both significantly lowered in STCM-NVs@DOX treated group compared to the control group (Fig. 7D). In addition, we assessed the infiltration of F4/80 tumor associated macrophages (TAMs) in tumor tissue through IHC staining for F4/80. The results revealed that STCM-NVs@DOX treatment substantially reduced tumor-infiltrating TAMs compared to other treatment groups (Fig. 7E and **Fig S4B**). These results imply that STCM-NVs@DOX treatment has the potential to alleviate tumor-associated immunosuppression. histological analysis of various major organs by H&E showed no obvious morphological changes or tissue damage were observed in heart, liver, lung, and kidney of mice following treatment with STCM-NVs@DOX (Fig. 7F). Taken together, these findings support the safety of STCM-NVs@DOX administration *in vivo*.

3. Discussion

To date, multiple studies have characterized a novel cannibalistic phenotype presented by chemotherapy-induced senescent tumor cell, whereby they actively localized to the adjacent healthy tumor cells and engulf them, as confirmed *in vivo* and *in vitro* [20–21]. However, healthy tumor cells usually do not actively contact and engulf their counterparts except for apoptotic tumor cells [42]. Additional studies have shown that when senescent cancer cells contact adjacent healthy cancer cells, their filopodial extensions attach tightly to the adjacent healthy cancer surface [20]. Consistently, we found that adjacent senescent cancer cells were connected via densely elongated filopodial extensions as shown by SEM method (Fig. 5A and Fig. 6I). Based on above findings, we speculate that the cell membrane surface of senescent tumor cells is enriched with specific recognition and cell adhesion molecules as well as unique physiological structure, thus facilitating targeted binding to homotypic cancer

cells in comparison to healthy cancer cells. Thereafter, we proposed a strategy to prepare a novel type of nanovesicle carrier by utilizing senescent tumor cell-derived cell membranes (STCM-NVs) for tumor site-targeted delivery drugs. According to our results, STCM-NVs indeed possess a remarkably higher and faster tumor targeting and retention efficiency (Fig. 2 and Fig. 4). They harness strong tumor target adhesion capacity and targeted cancer cells to initiate internalization process quickly and resist off target effect caused by FSS. The conventional approaches to enhance tumor-oriented distribution of CCM-based NVs involve chemical conjugation of ligands or adaptors to the CCM-NVs surface [14–16], but they usually increase immunogenicity and toxicity and compromise the integrity of CCM components and functions. However, the approach of preparing STCM-NVs was simple and controllable without any requirement of intricate and strenuous membrane modification process. The low cost of raw materials, stable performance and high yield make STCMs an ideal choice for convenient production of STCM-NVs, thus enabling a scale-up production of this novel delivery platform for pharmaceutical applications. In addition, current study has demonstrated the biocompatibility and safety of STCM-NVs, further highlighting their potential translational application.

Unlike conventional cell membrane-based nanovesicle, STCMs have rarely been considered as ideal drug deliver carriers due to their inadequate physical stability, making it prone to rupture and hard to protect the internal drug [43]. Moreover, a recent study has compared the uptake of extracellular vesicles from senescence tumor cells (S-EVs) and proliferating healthy tumor cells (P-EVs) [27]. and reported that P-EVs were readily taken up by proliferating healthy tumor cells (Hela cells) but S-EVs were not. The authors speculate that the high level of DPP4 protein on the surface of S-EVs make them refractory to internalization by proliferating healthy tumor cells. Conventionally, the surface proteome of EVs surface are similar to those of their parent cell membrane surface [44], At this point, the senescent tumor cell membrane was not an ideal carrier material. However, our precent study has demonstrated that the protein component on the EVs surface and homotypic cells membrane was different. As shown in **Fig S11**, DPP4 expression level didn't show a uniform upregulation in senescent tumor cells induced by varying concentration of DOX. Meanwhile, the expression of CD9 and CD81, exosome markers, in S-EVs group was significantly higher than those in homotypic senescent cancer cell group (MDA-MB-231). Of note, despite the elevated presence of DPP4 on the surface of STCM_S, it failed to inhibit the uptake of STCM-NVs by healthy cancer cells (Fig. 2A and **Fig S11**). Hence, the functions and characteristics of EVs surface could not be equated with those homologous cells' membrane, implying that healthy cancer cell uptake STCM-NVs and S-EVs through different mechanisms.

Our data further demonstrated that the STCM-NVs@DOX had a slightly longer retention time in the blood compared with HTCM-NVs@DOX (Fig. 3G). Meanwhile, *in vitro* study revealed that STCM-NVs@DOX exhibited a slower drug-release profile than HTCM-NVs@DOX at early stage (Fig. 3F). This could result from the freezing of phospholipid fatty acid tails in senescent cell membrane reduces cell fluidity by transitioning from the liquid to the gel phase, subsequently, decreases cell membrane permeability [45–46]. Taken together,

In order to elucidate the mechanism by which STCM-NVs were readily taken up by homotypic cancer cells compared to HTCM-NVs, it is important to establish how healthy cancer cells taken up STCM-EVs. In our current study, we found that pretreating healthy cancer cells with m β CD blocked the uptake of STCM-NVs (Fig. 5B). m β CD is a specific inhibitor for caveolae-mediated endocytosis pathway [36]. The characteristic of caveolae structure is a bulb-shaped pit covered with caveolins and cholesterol, serving to accommodate cargo and transport them into cell [47]. Notably, the interior of caveolae, around 50 nm in diameter, can only accommodate relatively small cargo [47], STCM-NVs prepared in our study was approximately 150 nm in diameter (Fig. 1G), therefore, it was not clear how the caveolae structure could accommodate materials with greater diameters than that of this pathway. Herein, we demonstrated the concentration and dissipating of F-actin at engulfing area of target cancer cell membrane were tightly correlated with STCM-NVs internalization (Fig. 5L, Fig S10). As known F-actin is capable of facilitating mechanical deformation of cell membrane [48]. Thus we speculate that the F-actin may enlarged the caveolae size to facilitate wrapping of larger particles (Fig. 5M). However, further studies are warranted to elucidate the molecular mechanisms in detail. Although several studies have identified various molecules that can be specifically recognized by cancer cells [49], the specific molecules that dictated the targeting and adhesion of STCM-NVs by homotypic healthy cancer cells remain largely unknown. One of the striking findings from our study is the identification of specific “recognize me” (FN1) and “eat me” (collagen) signals on STCM-NVs surface, which mechanistically explains their superior tumor targeting and cellular internalization at molecular level.

Further studies were then conducted to determine whether HTCM-NVs and STCM-NVs utilize distinct mechanism for tumor targeting and internalization. Our initial findings showed that the absence of FN1 in HTCM-NVs exhibited significantly decreased their adhesion to targeted cells, like the STCM-NVs performance (**Fig S6**). Nevertheless, no variations were observed in the internalization of HTCM-NVs after pretreating homotypic cancer cells with endocytosis pathway inhibitors CPZ, EIPA and M β CD, whereas the uptake of STCM-NVs was markedly suppressed by M β CD (**Fig S12**). Similarly, we found that the degradation of collagen in HTCM-NVs did not impact the uptake efficiency of these NVs by target cells, yet completely hindered the uptake of STCM-NVs (**Fig S13**). This observation indirectly demonstrated that compared to healthy cancer cells, the level of surface collagen proteins in senescent cancer cells induced by chemotherapy drug remained unchanged, while exhibiting notable structural and functional alterations. Together, these data suggest that the tumor targeting adhesion mechanisms exhibited similarities between STCM-NVs and HTCM-NVs. The enhanced tumor-targeting ability of STCM-NVs might be attributed to the high expression level of FN1 on the STCM_s surface, while the internalization mechanisms of STCM-NVs and HTCM-NVs by homotypic cancer cells were completely different.

To further assess the senescence-induced membrane remodeling strategy for amplifying the innate tumor-targeting capacity of cell-derived nanocarriers. We compared STCM-NVs from two kinds of breast cancer cell lines (MCF-7&MDA-MB-231) with different cannibalism frequencies. Notably, the comparable targeting efficacy observed between the two sources confirms that senescence-enhanced engulfment and tumor-targeting are functionally decoupled (Fig. 6). This decoupling supports a senescence-based

universal strategy for augmenting the intrinsic homing capacity of nanovesicles, irrespective of the inherent phagocytic variability across tumor cell types.

An interesting study indicate that tumor cells employed a resistance mechanism to eliminate internalized cargos from their surface [50]. Consequently, anti-cancer drugs were hindered from efficiently penetrating the cells and inducing their cytotoxic effect [50]. According to this point, we hypothesize that HTCM-NVs are recognized by healthy cancer cells as themselves. To ensure their survival, cancer cells might trigger resistance mechanism to protect themselves from cellular cannibalism. Since the protein profiles in STCM-NVs and HTCM-NVs were different, the homotypic cancer cells did not recognize STCM-NVs as certainly. However, further studies are warranted to demonstrated this hypothesis.

4. Conclusion

In summary, we ingeniously leveraged the cannibalistic properties of senescent cancer cells to develop a new type of drug delivery systems based on the unique homotypic tumor-targeting ability of senescent tumor cell membrane (STCMs). Using DOX as a model drug, STCM_S based nanovesicle loaded DOX (STCM-NVs@DOX) exhibited enhanced specific tumor-targeting delivery and therapeutic efficiency both *in vitro* and *in vivo* compared with those of free DOX and HTCM-NVs@DOX. Our findings support that the STCM-NVs hold promise to be a highly efficient tumor targeted drug delivery carrier, highlighting its potential translational applications to meet the increasing clinical demands.

5. Experimental Section

5.1. Cell culture and transfection

The culture conditions of human breast cancer cell and normal mammary epithelial cells MCF-10A are described in *SI Appendix, SI Text*.

5.2. Senescence assays

Senescence of MDA-MB-231 cells or MCF-7 cells were induced by treatment with 0.15 μ M DOX (44583, Merck) every 48 h, and senescent cells were harvested on day 6 or day 7. Senescence was evaluated by measuring SA- β -galactosidase activity (Cell Signal Technology) and by evaluating the expression levels of the senescent marker byvia qPCR and western blot.

5.3. The preparation of STCM-NVs@DOX

(1) Dil (near-infrared) (C1036, Beyotime) /DiD (far-infrared) (E607333-0010, Sangon) fluorescent dye solution was evenly applied to the surface of phoscent MDA- MB-231 cells. The cells were then placed in a CO₂ shaking incubator (oscillation amplitude: 50 mm, frequency: 30 rpm) and gently shaken in the dark at low speed for 30 min at 37°C. The dye solution was removed followed by washing twice with PBS. The pre-labeled cells were carefully scraped with a cell scraper. Cells were centrifuged at 800 rpm for 5 min

at 4°C, and the cell pellet was collected. (2) The cell pellet was resuspended in hypotonic lysis buffer at 50 µL per 5×10^6 cells and subjected to four freeze-thaw cycles in liquid nitrogen and a 37°C water bath, followed by intermittent sonication for 3 min with a probe sonicator at 30% power and centrifugation at 1,000 rpm for 5 min at 4°C to remove cells debris. The supernatant was further centrifuged at 3,500 rpm for 5 min at 4°C, followed by high-speed centrifugation at 100,000 g 4°C for 40 min. The supernatant was discarded, and the pellet was resuspended in PBS containing a protease inhibitor mixture. (3) The protein concentration of the supernatant was determined using a BCA protein assay kit (P006C, Beyotime). The supernatant was then diluted with PBS to 200 µg/mL, followed by mixing with DOX solution to obtain final DOX concentration ranging at 300 µM. (4) The mixture was sequentially extruded through 1 µM (10418704, Whatman), 0.4 µM (10417104, Whatman) and 0.2 µm (10417012, Whatman) polycarbonate porous membrane using a liposome extruder (610000, Avanti). It was then centrifuged at 100,000 g for 30 min at 4°C, and the pellet was harvested, resuspended in PBS and stored temporarily at -80°C.

5.4. Characterization of STCM-NVs

The particle size, size distribution, and zeta potential of the STCM-NVs were measured by dynamic light scattering and laser Doppler electrophoresis, respectively, using a Zetasizer Nano. The morphology of nanovesicle carrier was observed by transmission electron microscope (TEM). Briefly, STCM-NVs were resuspended in PBS at an appropriate concentration and mixed with an equal volume of 4% PFA. 15 µL of sample was placed onto carbon-coated copper grids (930326, Sigma Aldrich) and incubated at room temperature for 20 min. The grids were then washed with PBS, fixed on the 1% glutaraldehyde (G6257, Merck) at room temperature for 5 min, and washed with ddH₂O. The grid was stained with 2% phosphotungstic acid (155970, Sigma Aldrich) in the dark for 10 min. The excess stain was wicked away with filter paper, and the grids were air-dried at room temperature before being examined under a transmission electron microscope. The absorption spectrum was detected by UV-vis spectrophotometer.

5.5. *In vitro* targeted adhesion, cellular uptake and anticancer effect

To evaluate the adhesion force between STCM-NVs and target cells, 7×10^4 MDA-MB-231 cells were seeded onto glass coverslips. After complete cell attachment, the cells were incubated with 3 µg of labeled STCM-NVs or HTCM-NVs (control group) for 2 h. Upon observation of obvious nanovesicles aggregation on the cell surface, the coverslips were washed with PBS, fixed with 4% PFA, and placed in a T25 cell culture flask containing 4 mL of PBS under 60 rpm speed oscillation for 30 min. The glass coverslips were then stained with DAPI. Images were captured using a confocal laser microscope. The fluorescence intensity on the cell surface was analyzed to assess the adhesion force of STCM-NVs and HTCM-NVs to the tumor cell membrane. To detect cellular uptake efficiency mediated by STCM-NVs, 1×10^5 MDA-MB-231 cells were incubated with STCM-NVs or control group HTCM-NVs (3 µg/mL) in the dark for 4–6 h under static condition. The cells were then washed three times with PBS and fixed with 4% PFA at room temperature for 15 min. Nuclei were stained with DAPI solution in the dark for 10 min. Images were captured using a confocal laser microscope. To assess the *in vitro* antitumor efficacy of STCM-

NVs@DOX, we first co-cultured MDA-MB-231 cells with STCM-NVs@DOX or free DOX (both at 2.5 μ M) for 24, 48, and 72 h and detected the cell proliferation rate by using the CCK-8 assay (40203ES60, Yesen). And then, we seeded MDA-MB-231 cells in 6 well plates at the same density, followed by adding free dox or STCM-NVs@DOX or HTCM-NVs@DOX in different groups for further incubation for 48 h. The percentage of Annexin V⁺ cells following different treatments were analyzed by flow cytometry with a Annexin V-APC/DAPI apoptosis detection kit (E-CK-A117, Elabscience). Finally, the colony formation assay was used to evaluate anticancer effect of different drug carrier. MA-MB-231 cells seeded in the 6-well plate (1000/well) were treated with 2.5 μ M STCM-NVs@DOX, HTCM-NVs@DOX or free DOX for 14 days. After incubation, cell colonies were washed with PBS, fixed with 4% PFA, and stained with 0.5% crystal violet solution, then imaged and quantified.

5.6. *In vivo* specific targeting and anticancer effect

When the orthotopic breast tumors reached an average of 0.3–0.5 cm³, the mice were randomly divided into six groups (n = 5) and received tail vein injections of 200 μ L of PBS, free DOX, STCM-NVs, HTCM-NVs, STCM-NVs@DOX, or HTCM-NVs@DOX (all nanovesicles were fluorescently labeled with DID). At 0.5, 4, 12, 24, 48, and 72 h post-injection, animal fluorescence imaging was conducted using an *in vivo* imaging system (IVIS, PerkinElmer, USA) to monitor the biodistribution and tumor-targeting efficiency. To quantitatively evaluate the tumor targeting efficiency, *ex vivo* fluorescence imaging was performed at 24 h and 48 h post injection. Tumors and livers were collected and captured their fluorescence signals using IVIS spectrum system. Then using Image J software to measure the mean fluorescence intensity (MFI) of tumors and livers form equal-area after background subtraction. The tumor-to-liver ration was then calculated as $\frac{MFI_{tumor}}{MFI_{liver}}$ to represent tumor targeting efficiency. Subsequently, treatment was continued with seven injections over a 50-day period, during which body weight and tumor volume were regularly monitored. At the end of the study, the mice were sacrificed, and blood samples, tumors as well as major organs (heart, liver, spleen, lung, and kidney) were harvested for analysis. In addition, tumors and major organs were collected for *ex vivo* imaging using the same IVIS system. The tumor growth inhibition rate (TIR) was calculated as following: $TIR (\%) = (1 - \frac{RTV_{trate}}{RTV_{control}})$,

$RTV = \frac{V_t}{V_o}$ where V_t is volume at a given time point and V_o is the initial volume.

5.7. Endocytic pathway analysis

To investigate the mechanism of internalization under inhibition conditions, MDA-MB-231 cells were seeded into a 12-well plate and incubated overnight. The original medium was replaced with fresh medium containing either 10 μ M CPZ (HY-12708, MedChemExpres) for 2 h, 100 mM EIPA (HY-101840, MedChemExpres) for 48 h, or 0.05 μ M M β CD for 1 h, respectively. Subsequently, 20 μ g Dil labeled red fluorescent STCM-NVs or HTCM-NVs (diluted in 1 mL DMEM) was added into each well. After incubation for another 4–6 h at 37°C. The endocytic uptake of nanovesicles by cancer cells was observed using a confocal laser microscope.

5.8. Statistical analysis

All data are presented as mean ± standard error (SEM) from at least three independent experiments. Differences between experimental and control groups were analyzed by two-tailed unpaired Student's *t* tests using GraphPad Prism 9.0. Significance thresholds: ns (not significant) $p \geq 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Abbreviations

Abbreviations

Full term

HTCM-NVs

Healthy tumor cell membrane nanovesicles

STCM-NVs

Senescent tumor cell membrane nanovesicles

CCM

Cancer cell membrane

FN1

Fibronectin-1

CDH1

E-cadherin protein

COL

Collagen

PBS

Phosphate-buffered saline

EdU

5-Ethynyl-2'-deoxyuridine

DOX

Doxorubicin

TEM

Transmission electron microscopy

SEM

Scanning Electron Microscope

FSS

Flow shear stress

IHC

Immunohistochemistry

H&E

Hematoxylin and Eosin

CPZ

Chlorpromazine

EIPA

5-(N-ethyl-N-isopropyl)-amiloride

m β CD

Methyl- β -cyclodextrin

NTA

Nanoparticle tracking analysis

AST

Aspartate aminotransferase

ALT

Alanine aminotransferase

CREA

Creatinine

DID

1,1'-Diocetyl-3,3,3',3'-Tetramethylindodicarbocyanine,4-Chlorobenzenesulfonate Salt

Declarations

Ethics approval and consent to participate

The study involving all animal experiments were approved by the institutional animal care and use committee of Guangdong medical University (protocol No. GDY2602474).

Consent for publication

All the authors have agreed to publish this manuscript.

Competing interest

The authors declare no conflict of interest.

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declaration

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Author contributions

Liyuan Zheng: Conceptualization, Data curation, Formal analysis, Funding acquisition, Supervision, original draft Writing. **Shuxian Duan:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. **Yingying Li:** Methodology, Investigation, Visualization. **Che hui:** Data curation, Formal analysis, Methodology. **Yuran Liang:** Resources, Software, Visualization. **Xingding Zhang:** Resources. **Bihua Lin:** Project administration. **Caihong Li:** Resources. **Junjie Deng:** review and editing. **Xianxiu Qiu:** Resources. **Qunzhou Zhang:** review and editing. **Lin Qi:** Software. **Jinchen Zeng:** Funding acquisition, Supervision, review and editing.

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Availability of data and materials

The mass spectrometry proteomics data have been deposited to iProX database with project ID IPX0016960000 and all the other relevant data are available within the article and its supplementary information files.

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Scheme 1

Scheme 1 is available in the Supplementary Files section.

Figures

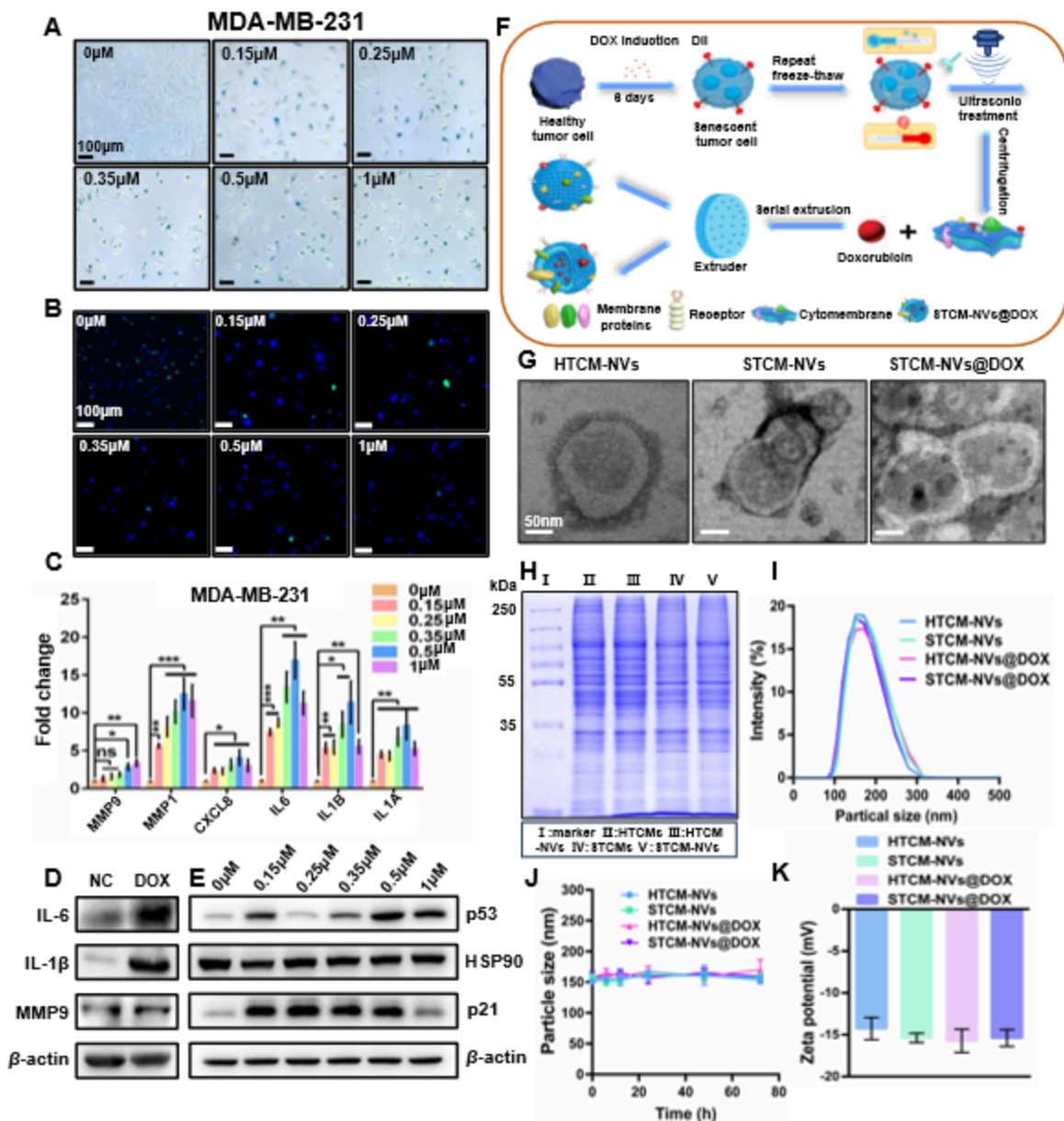


Figure 1

Induction of senescent MDA-MB-231 cells and characterization of STCM-NVs. (A) SA β -gal (green) staining of MDA-MB-231 cells following senescence induction by treatment with various concentration of DOX for 6 days. (B) EdU positive nuclei and Hoechst-stained nuclei of all the cells (blue) were visualized under a fluorescence microscope. (C) qRT-PCR analyses of mRNA level of a panel of SASPs, including MMP9, MMP1, IL-8, IL-6, IL-1 β and IL- α in MDA-MB-231 cells after senescence induction with 0.15-1 μ M DOX. (D) Western blot analysis of the protein expression level of IL-6, IL-1 β and MMP9 in MDA-MB-231 cells after senescence induction with 0.15 μ M DOX. (E) The levels of the senescence marker protein p53 and p21 in MDA-MB-231 cells after senescence induction with 0.15-1 μ M DOX. (F) Illustration of STCM-NVs@DOX preparation and tumor targeting therapy. (G) Representative images from transmission electron microscope (TEM) images showing the morphology of STCM-NVs, HTCM-NVs and STCM-NVs@DOX. (H) The membrane proteins contents in HTCM, STCM, STCM-NVs and STCM-

NVs@DOX were analyzed by using SDS-PAGE and Coomassie blue staining. (I) hydrodynamic size of HTCM-NVs, STCM-NVs, HTCM-NVs@DOX and STCM-NVs@DOX as measure by Nanosight NS300 Nanoparticle analysis (NTA, methods.) (J) The storage stability of STCM-NVs and STCM-NVs@DOX. (K) The zeta potential of different NVs were measured by dynamic light scattering. Data in C, I, J and K are the means $\pm$ SEM from three biological replicates; statistical analysis was performed by student's t test for two group comparison. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

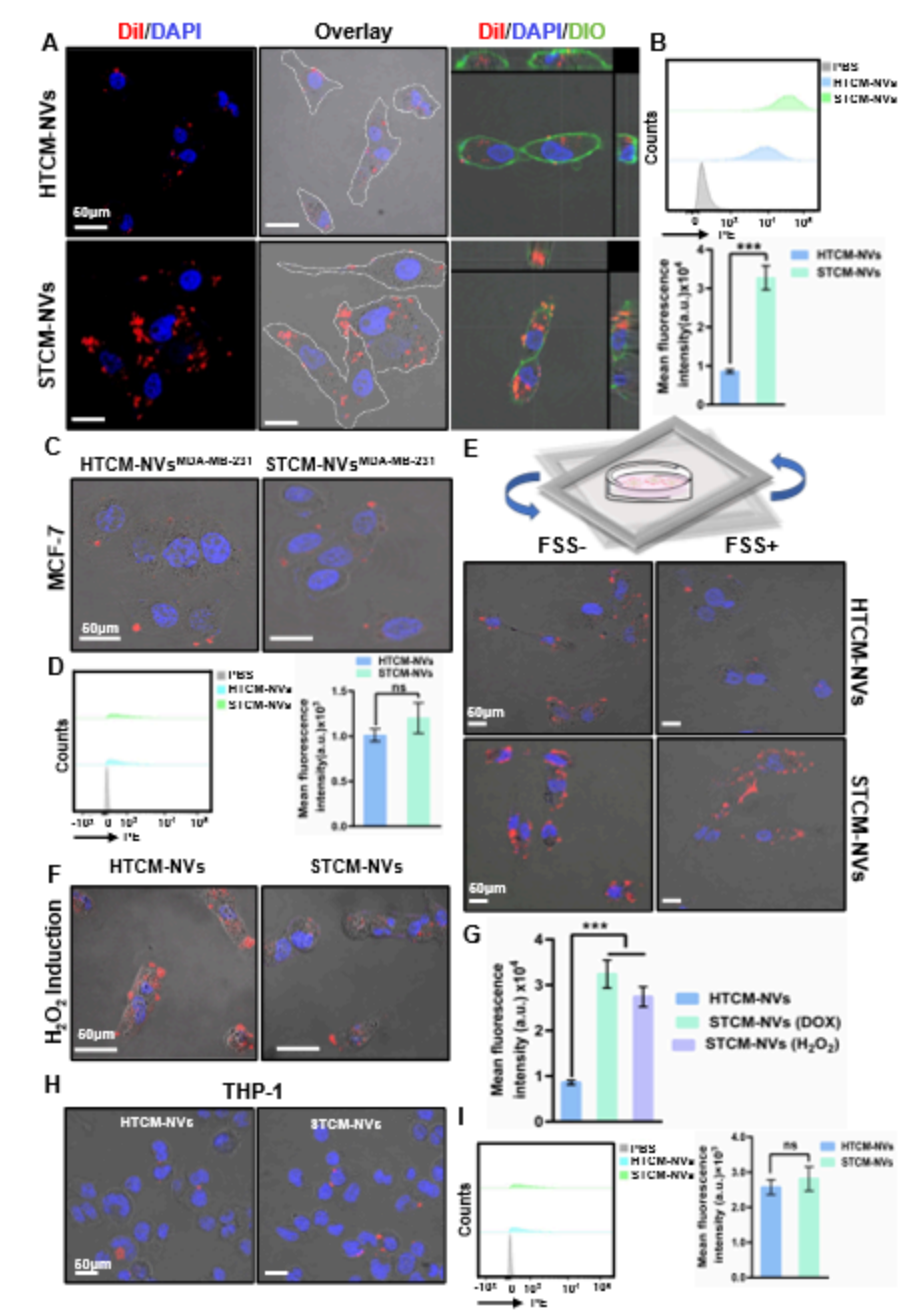


Figure 2

Enhanced targeted adhesion and cellular uptake of STCM-NVs in comparison to HTCM-NVs. (A) Fluorescence images of MDA-MB-231 cells after incubation with STCM-NVs or HTCM-NVs for 4 h and 37 °C. The orthogonal sections of MDA-MB-231 cell membrane labeled with DIO green, and NVs labeled

with Dil red were observed through laser confocal scanning microscope, (B) Uptake efficiency of STCM-NVs and HTCM-NVs after incubation for 4 h with MDA-MB-231 cells, measured by flow cytometry. (C-D) Heterotypic adhesion of STCM-NVs or HTCM-NVs was detected by Fluorescence images and flow cytometry. (E) Scheme of the fluid flow stimulation experiment. Fluorescence images show the homotypic adhesion of HTCM-NVs and STCM-NVs with or without FSS stimulation. (F) Fluorescence images analysis of cellular uptake of H₂O₂-induced STCM-NVs by homotypic cancer target cells. (G) Flow cytometric analysis of mean fluorescence intensity of various cell membrane NVs after co-incubation with homotypic cancer target cells. (H) The representative confocal microscope images of the uptake of HTCM-NVs and STCM-NVs by THP-1. (I) Flow cytometric analysis THP-1 internalized HTCM-NVs an STCM-NVs capacity. Data in B, D, and G are the means $\pm$ SEM from three biological replicates; Data were analyzed by student's *t* test. ****p* $\leq$ 0.001, ns, not significant.

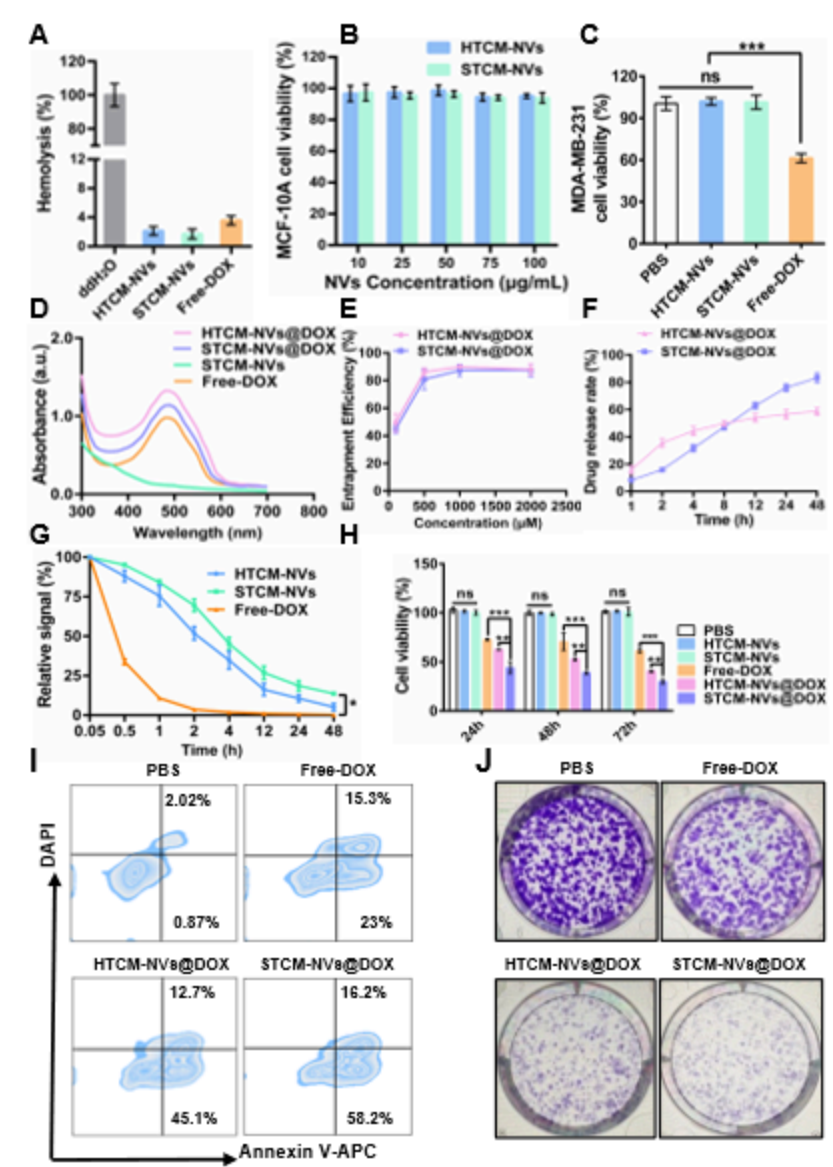


Figure 3

The properties STCM-NVs and anti-tumor effect of STCM-NVs@DOX *in vitro*. (A) The hemolysis percentage of red blood cells (RBCs) incubated with different NVs for 24 h at concentration of 100 $\mu\text{g/mL}$. Positive and negative controls are the RBCs in water and PBS, respectively. (B) Cytotoxicity of MCF-10A cells after being treated with STCM-NVs for 48 h at concentration of 10-100 $\mu\text{g/mL}$. (C) Following incubation of MDA-MB-231 with STCM-NVs for 24 h, cell viability was determined by CCK8 assay to assessment the correlation between STCM-NVs and the promotion of cancer risk. (D) UV absorption spectrum of STCM-NVs@DOX, STCM-NVs and DOX. (E) The entrapment efficiency of STCM-NVs@DOX and HTCM-NVs@DOX. (F) *In vitro* DOX release behavior from STCM-NVs@DOX and HTCM-NVs@DOX. (G) Pharmacokinetic profiles of STCM-NVs@DOX and HTCM-NVs@DOX after injection in to normal mice. (H) Cell viability of MDA-MB-231 cells following treatment with free DOX, HTCM-NVs@DOX and STCM-NVs@DOX for 1-3 days. Negative control groups were PBS and HTCM-NVs and STCM-NVs. (I) Flow cytometric analysis of apoptosis MDA-MB-231 cells after treatment with free DOX, HTCM-NVs@DOX and STCM-NVs@DOX for 48 h. (J) Colony formation assay was performed to detect the proliferation of MDA-MB-231 cells after treatment with different NVs. Negative control group was PBS. Data A-I are the means $\pm$ SEM of three biological replicates; student's *t*-test was used for two groups comparison. ** $p \leq 0.01$, *** $p \leq 0.001$; ns, not significant.

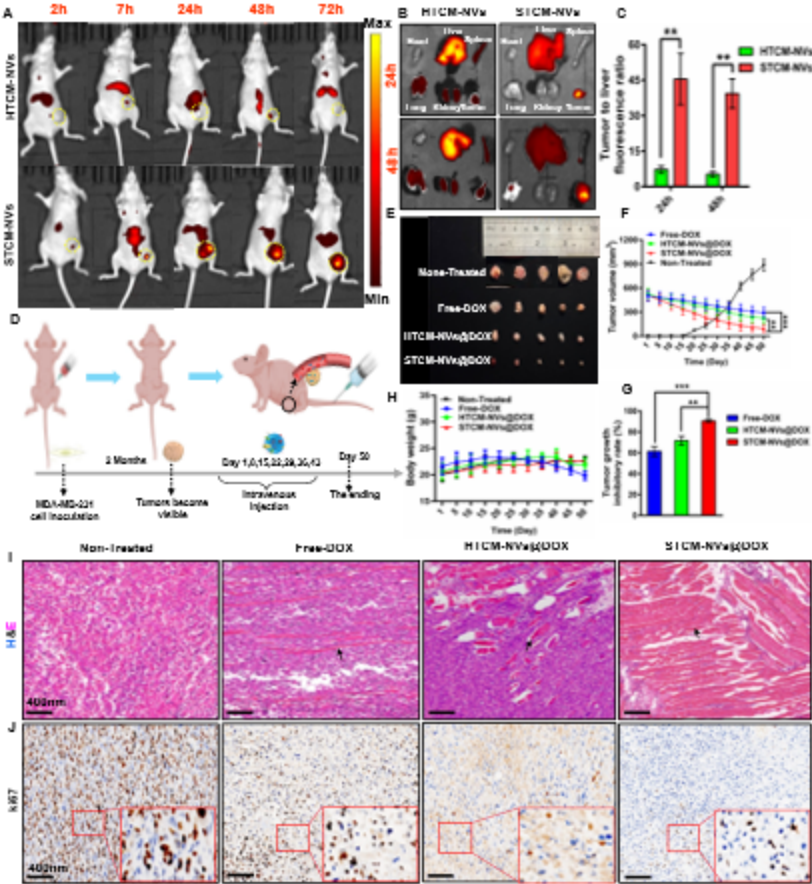


Figure 4

Evaluation of tumor-targeted delivery of STCM-NVs and anti-tumor efficacy of STCM-NVs@DOX *in vivo*. (A) The time distribution of STCM-NVs and HTCM-NVs in MDA-MB-231 tumor-bearing mice after injection through the tail vein (n=5 mice). DiD was a NIR fluorescence probe embedded in the cell membrane. The representative confocal microscope images of the uptake of HTCM-NVs and STCM-NVs. (B) The NIR spectrum images of distribution of STCM-NVs and HTCM-NVs in major organs after intravenous injection 24 h and 48 h (n=3 mice). (C) Semiquantitative analysis of ROI from tumor and liver in MDA-MB-231 tumor-bearing mice at 24 h and 48 h post injection. (D) Schematic of the experimental schedule of the MDA-MB-231 orthotopic breast cancer mouse model. (E) Optical images of intact tumor tissues of mice in each treatment group. (F) Relative tumor volume after different treatment, None-treated mice as control group. (G) Tumor inhibition rates calculated from tumor volumes at the endpoint. (H) Weight change curves of tumor-bearing mice post-treatment. (n=5 mice) (I) H&E examination of tumor at 50 days post one set of different treatments. (J) IHC staining for Ki67 in tumor tissues derive from different treatment groups. The values in E-G were presented by means $\pm$ SEM; 5-6 mice were included in each group. $**p \leq 0.01$, $***p \leq 0.001$ vs non-treated group.

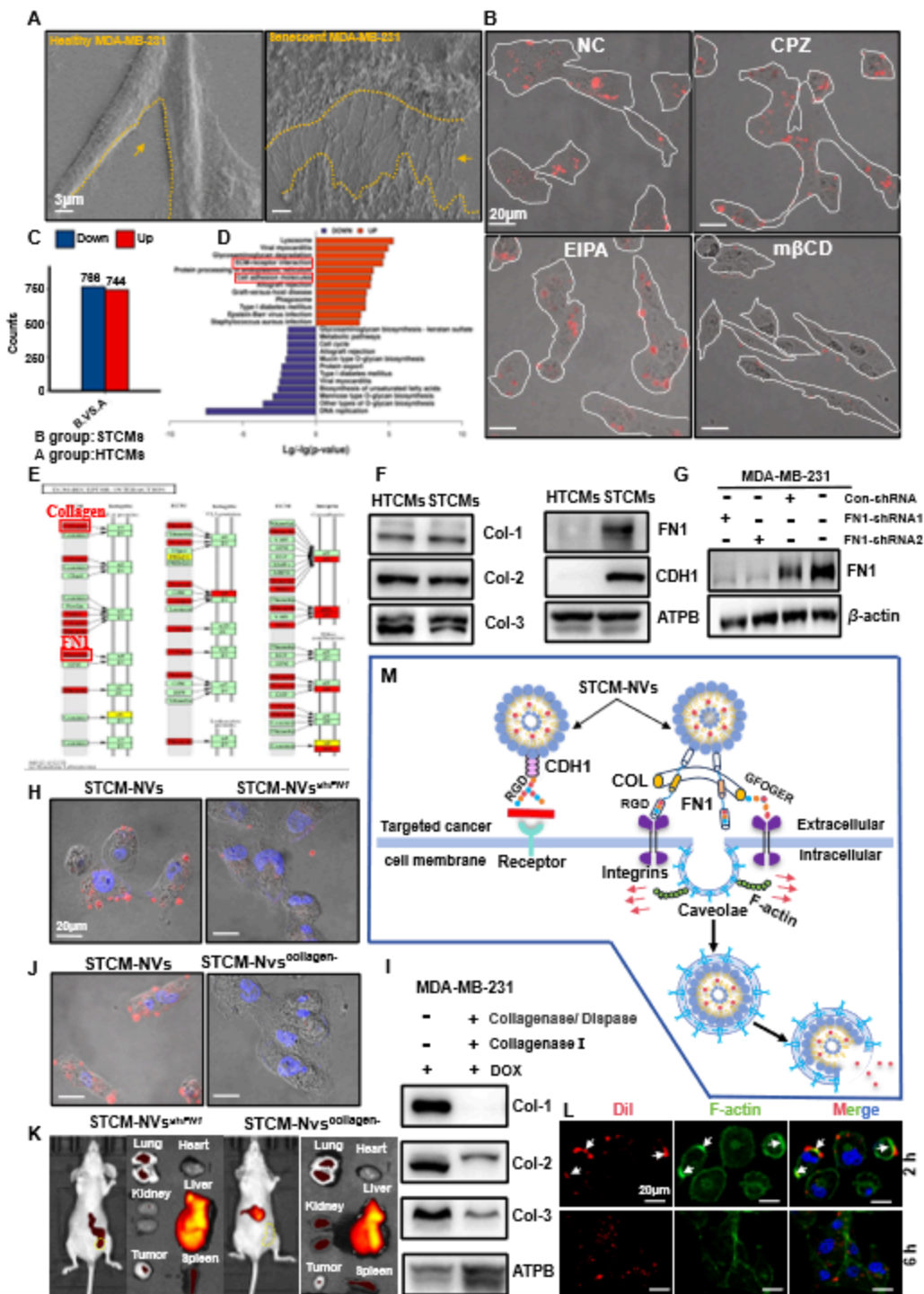


Figure 5

Investigation of the tumor targeted adhesion and internalization of STCM-NVs. (A) Representative SEM images showing the morphology of connections between neighboring healthy cancer cells or senescent cancer cells. (B) Cellular uptake of STCM-NVs by MDA-MB-231 (pretreated with CPZ, EIPA or MβCD) was visualized using laser confocal microscopy. (C) The bar chart of differential protein expression level in STCM_s versus and HTCM_s. (D) The KEGG pathway enrichment butterfly chart of up-regulated and down-regulated proteins in STCM_s vs HTCM_s. (E) The KEGG pathway diagram of ECM-receptor. Red filled

squares indicate up-regulated proteins, and green filled squares indicate background proteins. (F) Western blot analysis of the level of Col-1/-2/-3, FH1 and CDH1 on STCM_S and HTCM_S surface. (G) Knock down efficiency of FN1-shRNAs was analyzed by western blot in MDA-MB-231 cells. (H) Laser confocal microscopy analysis showing the decreased targeted adhesion between STCM-NVs^{shFN1} and homotypic cancer cells after co-incubation for 2 h. (I) The degradation efficiency of collagens was measured by western blot. (J) Representative fluorescence images showed significantly reduced uptake efficiency of STCM-NVs^{collagen-} by homotypic cancer cells. (K) The distribution of Dil labeled STCM-NVs^{shFN1} and STCM-NVs^{collagen-} in MDA-MB-231 tumor-bearing mice and major organs after intravenous injection 24 h (n=3 mice). (L) Confocal imaging shown the dynamic concentration and disappearance of F-actin in target cell during the early and late stages of engulfment. Concentrated area of F-actin are indicated by white arrow. (M) Schematic diagram of the specific homologous targeting ability and highly efficient internalization mechanism of STCM-NVs.

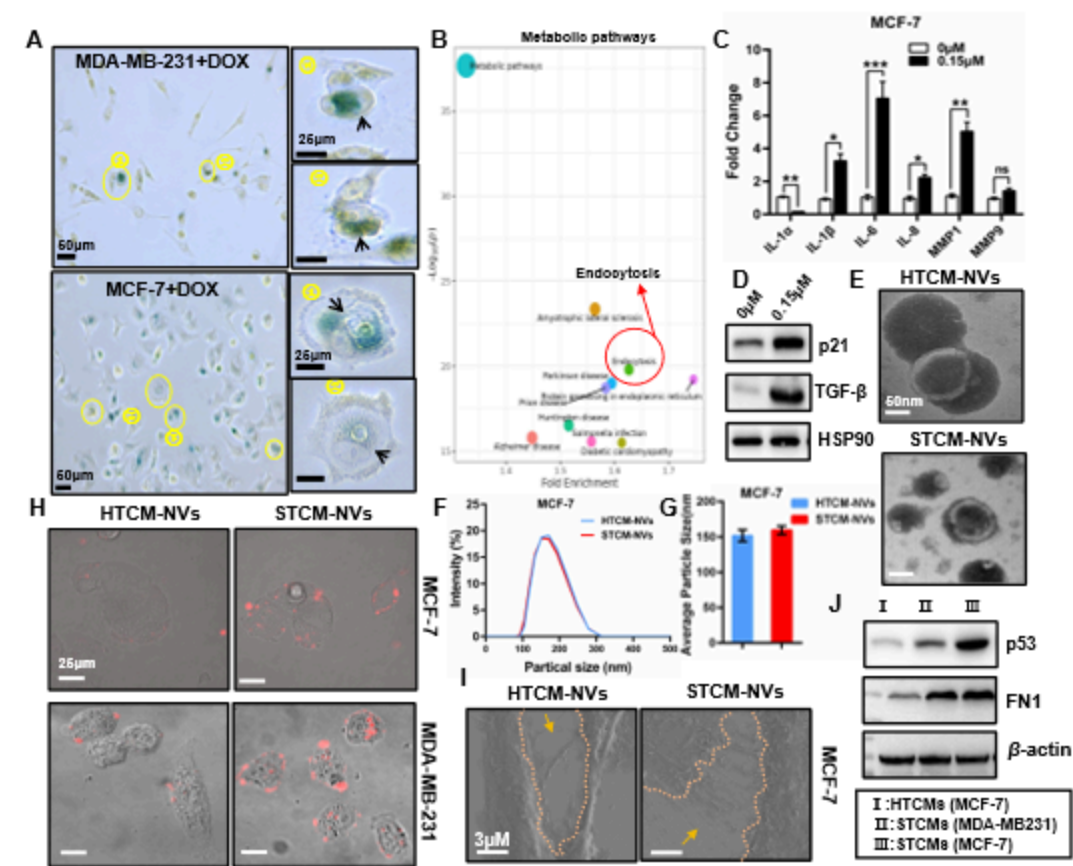


Figure 6

Effect of cannibalism frequency on the native tumor targeting capacity of STCM-NVs. (A) SA-βGAL staining images in color bright field. Circles and Arrowheads indicate engulfed cells visible in images. (B) KEGG pathway analysis of differential protein enrichment in endocytic pathways upon senescence in MDA-MB-231 cells. (C) qRT-PCR analyses of mRNA levels of a panel of SASPs, including MMP9, MMP1, IL-8, IL-6, IL-1β and IL-α in MCF-7 cells after senescence induction with 0.15 μM DOX. (D) Western blot analysis of the protein expression level of TGF-β and p21 in MCF-7 cells after senescence induction with

0.15 μM DOX. (E) Representative transmission electron microscopy (TEM) images showing the morphology of STCM-NVs from MCF-7 cells. (F-G) The hydrodynamic size of HTCM-NVs and STCM-NVs from MCF-7 as measured by Nanosight NS300 nanoparticle analysis (NTA). (H) Representative confocal microscopy images of STCM-NVs and HTCM-NVs binding to homologous cancer cells. (I) Representative SEM images showing the morphology of connections between neighboring healthy MCF-7 cells or senescent MCF-7 cells. (J) Western blot analysis of the proteins level of p53 and FN1 in different cancer cells. Data in (C) is expressed as mean $\pm$ SEM from three biological replicates; statistical analysis was performed by student's t test for two group comparison. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ns, not significant.

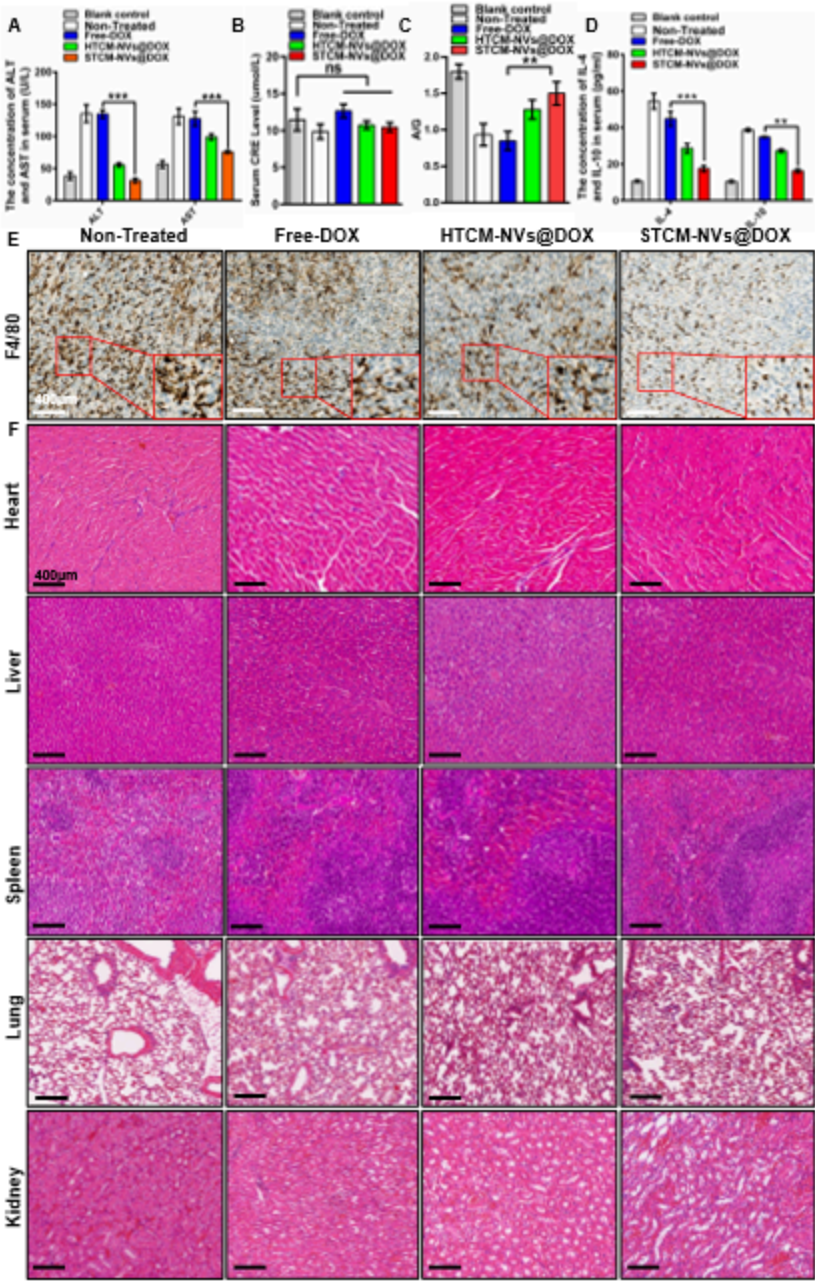


Figure 7

***In vivo* biosafety assessment.** (A-D) Blood biochemical analysis after 50 days of treatment with free DOX, HTCM-NVs@DOX and STCM-NVs@DOX, showing insignificant toxicity over the various parameters. (ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRE: creatinine, A/G: albumin/globulin ratio. IL-4 and IL-10 were classic anti-inflammatory factors). (E) IHC staining for F4/80 macrophages in tumor tissues derive from different treatment groups. (F) H & E staining of paraffin-embedded sections of the heart, liver, spleen, lung and kidney from mice in each treatment group. Data A-D are shown as means $\pm$ S.E.M of three biological replicates; student's *t*-test was used to assess significance in two-group comparisons, $**p \leq 0.01$, $***p \leq 0.001$; ns, not significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [HTCMNVs.avi](#)
- [STCMNVs1.avi](#)
- [Supplementaryinformation.docx](#)
- [floatimage1.png](#)
- [Scheme1.docx](#)