Virion-Like Membrane-Breaking Nanoparticles with Tumor-Activated Cell-and-Tissue Dual-Penetration Conquer Impermeable Cancer

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Poor drug penetration into tumor cells and tissues is a worldwide difficulty in cancer therapy. A strategy is developed for virion-like membrane-breaking nanoparticles (MBNs) to smoothly accomplish tumor-activated cell-and-tissue dual-penetration for surmounting impermeable drug-resistant cancer. Tailor-made dendritic arginine-rich peptide prodrugs are designed to mimic viral protein transduction domains and globular protein architectures. Attractively, these protein mimics self-assemble into virion-like nanoparticles in aqueous solution, having highly ordered secondary structure. Tumor-specific acidity conditions would activate the membrane-breaking ability of these virion-like nanoparticles to perforate artificial and natural membrane systems. As expected, MBNs achieve highly efficient drug penetration into drug-resistant human ovarian (SKOV3/R) cancer cells. Most importantly, the well-organized MBNs can pass through endothelial/tumor cells and spread from one cell to another one. Intravenous injection of MBNs into nude mice bearing impermeable SKOV3/R tumors suggests that the MBNs can recognize the tumor tissue after prolonged blood circulation, evoke the membrane-breaking function for robust transvascular extravasation, and penetrate into the deep tumor tissue. This work provides the first demonstration of sophisticated molecular and supramolecular engineering of virion-like MBNs to realize the long-awaited cell-and-tissue dual-penetration, contributing to the development of a brand-new avenue for dealing with incurable cancers.

A major obstacle to cancer chemotherapy is that only a very small part of systemically administered molecules reaches designated tumor tissues and cells, leading to treatment failure and even multidrug resistance.[1] A variety of nanocarriers have marginally improved drug pharmacokinetics and tumor accumulation, whereas some severe biological barriers including tissue and cell barriers urgently need to be overcome for perfect chemotherapeutic delivery,[2] especially for drug-resistant or impermeable cancers (e.g., human pancreatic adenocarcinoma and human ovarian carcinoma).[3] Small-molecule drugs and most nanocarriers are difficult to achieve efficient tissue-and-cell penetration,[4]; hence, more attention is paid to fabricate robust delivery system for deep tumor penetration and cellular internalization.[5] Inspired by viral protein transduction domains (such as HIV transcription (Tat) proteins and HSV-1 VP22 proteins),[6] many naturally occurring penetrating peptides have been exploited for promoting drug-loaded nanoparticles across cell membrane barriers.[7] Excitingly, recent findings suggest that tailoring peptide sequence with a key R/KXXR/K motif binding to neuropilin-1 can provide desirable tissue-penetrating activity,[8] facilitating deep targeted delivery of nanoparticles within the solid tumors.[9] Nevertheless, up to now, very few penetrating peptides are able to synchronously facilitate cell-and-tissue penetration of nanoparticles within solid tumor.[10] As a result, developing innovative peptide strategy on tumor-specific tissue-and-cell dual-penetration for nanoparticle transportation will hopefully make scientific conception of “magic bullet” treatment a reality, completely improving the therapeutic index of cancer treatment.

Natural virion nanoparticles are known to powerfully infect specific tissues and organs in organisms with two critical steps of rapid target cell entry and efficient tissue spread.[11] Although researchers have successfully invented a series of artificial cell-penetrating peptides (such as polyarginines peptides, Pep-1 and transportan) through mimicking the pivotal sequence of above-mentioned viral protein transduction domains,[5a,12] current penetrating peptides fail to assist the nanoparticles permeate in tissue as virus infection from one cell to another cell. In addition to protein transduction domains, tissue-penetrable viroions always have highly ordered assembled nanoarchitectures for breaking the host cell membranes as well as invading surrounding cells.[11c,13] As a reference, peptide-assembled nanoparticles containing abundant cell-penetrating domains (including Tat and polyarginines R6) indeed are able to travel through microvascular endothelial cells of the blood–brain barrier.[14] For these reasons, creating a first dual-penetration...
strategy on virion-inspired peptide constructions, which not only can realize robust cell entry but also can vigorously penetrate into deep tissue, is a challenging and promising exploration for solving worldwide difficulties in anticancer therapy (particularly impermeable and multidrug-resistant tumors).

Herein, we demonstrate a synergistically molecular and supramolecular construction on the development of virion-like nanoparticles for guiding highly efficient chemotherapeutic penetration both in cells and tissues, as well as defeating impermeable multidrug-resistant tumors (Scheme 1). For mimicking the arginine(R)-rich domains in natural transduction peptides,[6a,7] dendritic arginine-rich architecture is designed to bring inherent multivalent effects to enhance penetrating ability,[15] due to optimized water solubility and cell membrane association.[16] Meanwhile, dendritic peptides are unique protein mimics for building virion-like nanostructures,[15a] such as our reporting artificial capsid.[17] On the other hand, hydrophobic antitumor drugs can be introduced into the peptide dendrons via dynamic chemical bonds to acquire dendritic peptide prodrugs with the following expectations[11a,18]: supramolecular interactions (such as hydrophobic effects and π stacking) drive the formation of highly ordered virion-like nanoarchitectures, and these virion-like nanoparticles validly harbor the drugs during the biological transportation but deliver these payloads with tumor-specific stimulation. The fluorescent drug of doxorubicin (DOX) is conjugated onto the core of dendritic peptides using acid-cleavable hydrazone bond in this study. The supramolecular virion-like nanoparticles are expected to punch pores on tumor cell membrane as natural membrane-breaking viruses,[19] as well as provide efficient cell and tissue penetration.[11a,b] More importantly, only if natural virions reach targeted site, host tissues and cells would launch intense viral infection.[11a] For instance, many common viruses (such as influenza viruses and alphaviruses) start membrane fusion and invasion after exposure to target cell with specific trigger (e.g., enzyme and low pH).[11c,13] Hence, endowing the virion-like nanoparticles with tumor-activatable dual-penetration must be an extremely important consideration to enhance tumor-specific invasion and minimize disturbance to normal tissue.

To conceal the penetrating ability during in vivo transportation, these virion-like nanoparticles are dressed with tumor-shred-dable masking (tumor microenvironment pH activation) in this design. We can predict that the virion-like nanoparticles would seek tumor site with blood circulation, recognize tumor microenvironment to activate penetrating capacity, break through vascular and tumor cells, permeate into deep solid tumor, and finally defeat impermeable cancer.

The viral building blocks of tumor-activated arginine-rich dendritic peptide prodrugs were accurately synthesized (Schemes S1 and S2, Supporting Information), and the details can be found in the Supporting Information. Briefly, we first prepared arginine-rich peptide dendrons using a divergent approach,[20] and dendritic core was decorated with the hydrazine hydrate for dynamic conjugation of hydrophobic drug
The arginine-rich dendritic peptide prodrugs had precise structure (protonated molecular weight, $M_w = 1566.9$) by matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF MS, Figure S7, Supporting Information), in good agreement with calculated value ($M_w = 1566.9$). Subsequently, peripheral arginine residues on dendritic prodrugs were masked with 2, 3-dimethylmaleic anhydride (DA) to obtain acid-activated dendritic peptide prodrugs (DA-Dend-R4K2K-Hyd-Drug, Figure S8, Supporting Information), and the purity could reach up to 99.9% (Figure S9, Supporting Information). The chemical characterizations for each compound were illustrated in Figures S1 to S8 in the Supporting Information. Then, mass spectra provided direct evidences to demonstrate tumor-activated features of dendritic peptide prodrugs (Figure 1A), including i) weakly acid condition analogous to tumor acid microenvironment (pH 6.5) triggered the cleavage of shielding groups to expose arginine-rich residues, and ii) intracellular lower pH condition of acid vesicular organelles (pH 5.0) induced the break of hydrazone bonds to release native drugs.

The amphiphilic dendritic peptide prodrugs would self-assemble into the well-defined nanostructures as shown in the transmission electron microscopy (TEM) image (Figure 1B), possessing highly ordered secondary structures by circular dichroism
(CD) detection (12.4% α-helix, 59.2% β-sheet, and 17.7% β-turn, Table S1, Supporting Information). These virus-inspired nanoparticles had an average size of 136.3 ± 5.8 nm and zeta potential of -29.8 mV (Figure 1C). Acid condition (pH 6.5) corresponding to tumor microenvironment activated the virus-like nanoparticles with a dramatic transformation of surface charge from negative to positive (+18.4 mV) due to the deshielding of stealth shells, but the nanoparticles still maintained well-defined nanostructure and ordered secondary structure to exert biological functions (Figure S12, Supporting Information). These virus-like nanoparticles could keep their stable nanostructures at pH 6.5 and 7.4 phosphate buffered solutions (PBS) which respectively mimic tumor extracellular and cytoplasmic pH conditions (Figure S15, Supporting Information).[21] as well as in the presence of serum (Figure S16, Supporting Information). Moreover, the arginine residues on virion-like nanoparticles were rapidly exposed at pH 6.5 (Figure 1D), while the DA-masked arginine residues kept stealthy status without amino exposure at pH 7.4 corresponding to normal physiological condition in systemic transportation. Additionally, lower pH condition (pH 5.0) of acid organelles (e.g., lysosome) induced the disintegration of nanoparticles and disappearance of typical secondary structure to release drugs (CD spectrum is similar to the result of DOX/peptide dendron mixture, Figure S14, Supporting Information). The cleavage of hydrazone bonds for DOX release was summarized in the release profiles with different pH conditions (Figure S17, Supporting Information). We also performed some basic properties of other control nanoparticles (Figures S18 to S20, Supporting Information); for example, inert arginine-rich nanoparticles assembled from SA-Dend-R4K2K-Hyd-Drug still hold a negative zeta potential without amino exposure under pH 7.4 and 6.5. In general, we successfully fabricated the virion-like nanoparticles with acid-activated arginine-rich corona.

To investigate the acid-activated virion-like nanoparticles for transmembrane, we visualized pH-dependent transportation across membrane using a convective model system. A biomimetic biphasic system is composed of top aqueous buffers (pH 7.4 or 6.5) and bottom chloroform phase containing 1% acidic lipids of lauric acid (LA), cholesterol sulfate (CS), or palmitoyl-oleoyl phosphatidyl-glycerol (POPG), respectively, which highly imitate the abundant carboxylates, phosphates, and sulfates in cell membrane constituents.[16] As shown in the Figure 1E, the unactivated nanoparticles with DOX moiety are always located in the buffer solution at a normal physiological pH 7.4, while low pH 6.5 remarkably activated transportation of nanoparticles across the intact interface into hydrophobic chloroform phase within a very short time (10 min), which should be attributed to virion-like nanostructures with abundant exposed arginine residues. Quantitative results for nanoparticle distribution also demonstrated that activated nanoparticles (pH 6.5) rapidly transferred into organic phase, while the unactivated nanoparticles still mainly partitioned in the buffer solution. In addition, the inert arginine-rich nanoparticles and lysine-rich nanoparticles showed poor ability on transportation across bulk water/chloroform interfaces (Figures S24 to S28, Supporting Information). Thus, it can be seen that the molecular and supramolecular engineering on virion-like arginine-rich nanoparticles succeeded in generating strong supramolecular interactions with biomimetic anionic lipids via multiple hydrogen bonding, indicating the great potentials on membrane perforations.

Next, we made use of mouse red blood cells (RBCs) to study acid-associated interactions of virion-like nanoparticles with natural membrane system. Visual and quantitative results of hemolysis assay were presented in Figure 1F. Under the physiological condition (pH 7.4), no hemolysis was observed and detected even at a high concentration of virion-like nanoparticles (100 µg mL⁻¹), implying that the nanoparticles were stealthily during the blood transportation and biocompatible to normal tissue. Nevertheless, the acid-activated nanoparticles caused significant hemolysis at a relatively low condition (10 µg mL⁻¹), suggesting powerful membrane-breaking activities to induce the release of hemoglobin. In the meantime, it was found that lysine-rich nanoparticles showed undesirable ability to trigger the rupture of RBCs at pH 7.4 and 6.5 (Figures S31 and S32, Supporting Information), which was in good consistent with their transportation in bulk water/chloroform mixture and some previous reports. And arginine-rich dendritic peptides (Dend-R4K2K) also failed to destroy RBCs at the concentration of 100 µg mL⁻¹ (Figures S33 and S34, Supporting Information). Moreover, scanning electron microscopy (SEM) studies confirmed that the unactivated nanoparticles have no obvious influence on the biconcave disc-like morphology of RBCs, but acid activation of nanoparticles resulted in prominent shape destroy and distortion of RBCs. Therefore, it concluded that both arginine-rich dendritic molecular engineering and virus-like supramolecular fabrication are vital to membrane-breaking capacity of nanoparticle design.

To further evaluate membrane-breaking potentials, drug-resistant human ovarian (SKOV3/R) cancer cells were used as a representative tumor cell line because of their hard membrane barriers and impermeable tumor tissue (including highly compact tumor interstitium, low vascular density, and small vessel diameter).[18] As shown in the SEM image (Figure 1G), SKOV3/R cells still possessed excellent spreading form and intact membrane after treatment with virion-like nanoparticles (10 µg mL⁻¹) at pH 7.4 culture medium, without differences of untreated SKOV3/R cells (Figures S37 and S38, Supporting Information). In contrast, SKOV3/R cells that were incubated with the acid-activated virion-like nanoparticles presented irregular morphology and spongy structure. Enlarged view of SEM image demonstrated apparent disruption on membrane ultra-structures with abundant perforations. The TEM image also displayed the acid-activated virion-like nanoparticles damaged membrane, resulting in discontinuous and fuzzy boundary (red arrows). More importantly, treatment with acid-activated virion-like nanoparticles led to a fast leakage of cytoplasmic lactate dehydrogenase (LDH) from tumor cells, testifying lesion and disintegration of cell membrane systems. By the way, reduction of intracellular LDH level contributed to accelerating the tumor death and overcoming drug resistance. As predicted, Dend-R4K2K, lysine-rich nanoparticles and inert arginine-rich nanoparticles failed to break drug-resistant cancer cell membrane at the same situation (pH 6.5, 10 µg mL⁻¹; Figures S39 to S41, Supporting Information). Taken together, tumor-activated virus-like nanoparticles with arginine-rich architecture could efficiently transport across tumor membrane and cause the marked membrane destruction at pH 6.5 condition mimicking...
tumor microenvironment, which was ascribed to the ultrastrong capacity on formation of multiple hydrogen bonding and numerous pores in tumor membranes.\[^{[19]}\] Therefore, these virion-like nanoparticles were termed as tumor-specific membrane-breaking nanoparticles (MBNs).

Encouraged by the potent damage capability on cell membrane barriers, we studied the utility of virion-like MBNs for improving cell penetration and drug internalization based on their inherent fluorescence. The SKOV3/R cells were treated with MBNs at a concentration of 10 µg mL\(^{-1}\) (3.8 × 10\(^{-6}\) m, containing 2 µg mL\(^{-1}\) DOX). As predicted, quantitative intracellular drug content by a flow cytometry measurement (Figure 2A) manifested that the acid-activated MBNs at pH 6.5 provided the ultrastrong ability to enhance drug penetration with 100% positive cell and \(\approx 10^5\) mean fluorescence intensity (MFI) value, much better than positive control of doxorubicin hydrochloride (DOX.HCl, 37% positive cell and \(\approx 10^3\) MFI value, Figure S43, Supporting Information). The MBNs at pH 7.4 without cell-penetrating potential showed very poor drug intake to drug-resistant tumor cells. Then, the real-time processes of drug penetration were intuitively monitored by a live cell station (Figure 2B). Little DOX fluorescence was captured in SKOV3/R cells during the incubation with MBNs at pH 7.4 medium for 24 h (Video S1, Supporting Information), whereas very strong drug fluorescence was observed in the acid-activated MBN group (pH 6.5) within half hour (Video S2, Supporting Information). Such fast and efficient drug penetration after tumor microenvironment pH activation revealed that our MBNs readily surmounted the main biological membrane barriers of tumor cells, allowing for elevating drug bioavailability and overcoming drug resistance. The hidden membrane-breaking activity via the negative shielding at the normal physical condition was capable of avoiding the invasion to normal cell/tissue and optimizing systemic transportation.

To further confirm membrane-breaking internalization, we used certain inhibitors to block endocytosis pathways of SKOV3/R cells. There was no impact on MBN internalization with pretreatment of nystatin (caveolin endocytosis inhibitor), chlorpromazine (clathrin endocytosis inhibitor), cytochalasin D (macropinocytosis inhibitors), and monensin (clathrin- and caveolin-independent endocytosis inhibitor), thereby we can infer that internalization of acid-activated MBNs is completely independent of specific endocytosis pathways (Figure 2C). Likewise, with energy suppression by NaN\(_3\), the acid-activated MBNs were still largely taken into SKOV3/R cells. A modest reduction of DOX internalization at 4 °C may be attributed to the restriction of membrane fluidity at lower temperature. However, the cellular uptake of lysine-rich nanoparticles largely depended on receptor-mediated endocytosis pathway (Figure S53, Supporting Information).

**Figure 2.** Robust cell penetration of MBNs. A) Quantitative drug penetration into SKOV3/R cells after incubation with DOX, DOX.HCl, and MBNs at pH 6.5 for 2 h with a drug concentration of 2 µg mL\(^{-1}\) by FACS detection. Treatment with MBNs at pH 7.4 was a control. B) Real-time live-cell images of SKOV3/R cells incubated with MBNs at pH 7.4 and 6.5, merging of MBNs channel (red) and light field. C) The relative drug internalization of MBNs at a DOX concentration of 2 µg mL\(^{-1}\) toward SKOV3/R cells at pH 6.5 after pretreated with endocytosis inhibitors, energy suppression (NaN\(_3\)), and low temperature (4 °C) as analyzed by microplate reader (\(n = 6\)). D) CLSM images and the line scanning profiles for SKOV3/R cells with or without bafilomycin (Baf, lysosomal inhibitor, 20 × 10\(^{-9}\) m, 30 min pretreatment) at pH 6.5 after 5 min incubation with MBNs, including DOX channel (red), LysoTracker-stained lysosome channel (blue), light field, and overlay images.
Figure 3. Deep tumor penetration of MBNs. A) Schematic illustration for in vitro experimental models of transvascular extravasation and intratumoral penetration. CLSM images and cell viability of cells in the transwell filter and the low chamber. Cell viability was detected by CCK-8 assay (n = 6). B) CLSM images of SKOV3/R MTS after 4 h incubation with MBNs at pH 7.4 or 6.5 (2 μg mL⁻¹ DOX). C) Schematic illustration and 3D-reconstructed CLSM images for SKOV3/R cells-contained matrigel in μ-slide plate treated with MBNs at pH 7.4 and 6.5 culture medium (2 μg mL⁻¹ DOX) including overlay image, DOX channel, and Hoechst 33342-stained nucleus channel. D) CLSM images of cryosections of SKOV3/R tumor after intravenous administration of MBNs and I-MBNs for 24 h (10 mg DOX kg⁻¹), including DAPI-stained nucleus channel (a, blue), FITC-CD31-stained blood vessel channel (b, green), DOX channel (c, red), and overlay image (d).
Afterward, we inspected intracellular MBN distribution with LysoTracker-stained channel using confocal laser scanning microscopy (CLSM). After incubation with the MBNs (10 µg mL⁻¹) at pH 6.5 for 5 min, red fluorescence signal of MBNs widely distributed in the whole SKOV3/R cells, rather than colocalization with endocytic organelles (Figure 2D). Furthermore, in the presence of bafilomycin (Baf) which efficiently inhibits endosomal acidification and escape, the uniform distribution MBNs also clearly appeared in the CLSM image similar to the untreated group, supporting that MBNs directly entered tumor cell by membrane perforation. Along with the extending of incubating time, MBNs would damage subcellular membrane systems of acid compartments (such as endosomes and lysosomes), as proved by no longer staining with LysoTracker (Figure S54, Supporting Information). Then, the interaction and damage of acid compartments could trigger the cleavage of hydrazone bond, which contributed to nucleus delivery of DOX to act pharmacological activity (Figures S55 and S56, Supporting Information). These results collectively manifested that the acid-activated virus-like nanoparticles with arginine-rich corona provided a much-desired tumor-specific membrane-breaking activity for highly efficient cell penetration and drug internalization.

Due to prominent membrane-breaking ability of the virus-like nanoparticles, we speculated that MBNs may strongly permeate tumor tissue through one-by-one cell perforation. Firstly, we established two Transwell models to explore the MBN permeability from vascular to tumor cells, as well as from outer cells to deeper cells (Figure 3A). A monolayer of endothelial (HUVEC) cells was seeded in Transwell insert to simulate the vascular barrier at tumor tissue. The HUVEC cells in the insert were treated with MBNs at pH 6.5 culture media for 2 h at a DOX concentration of 20 µg mL⁻¹, and then the media were replaced with fresh media and coincubated with SKOV3/R cells at the lower chamber for 24 h to study MBN translocation from endothelial cells to bottom tumor cells. The CLSM images revealed that the HUVEC cells hold strong red fluorescence of MBNs, and moreover apparent MBN fluorescence was distinctly observed in the lower chamber. This phenomenon indicated that a portion of MBNs broke through membrane barriers of monolayer endothelial cells and penetrated into SKOV3/R tumor cells in the lower chamber, attributing to the stable nanostructures of virion-like MBNs at the biomimetic tumor intracellular condition (serum-contained solution and pH 7.4 PBS) for efficient transvascular delivery. In the meantime, we detected the high cytotoxicity to both the top endothelial cells and bottom tumor cells by cell counting kit-8 (CCK-8) assay, suggesting that the acid-activated virus-like MBNs successfully traversed through endothelial cells and penetrated into SKOV3/R tumor cells to exert antitumor activity. For another, we seeded the SKOV3/R cells in Transwell insert and lower chamber to investigate the potential on intratumoral penetration. It was also concluded that MBNs could efficiently transport from one tumor cell to another tumor cell, as evidenced by high intracellular MBN fluorescence and low cell viability (~35%) of SKOV3/R cells at the lower chamber in Transwell model. Meanwhile, we constructed fluorescently labeled MBNs based on the DA/Cy5.5-Dend-R3K2K-Hyd-Drug (peptide dendron segment was labeled with Cy5.5). Colocation of DOX and Cy5.5 fluorescence within SKOV3/R tumor cells in low chamber at pH 6.5 (Figure S60, Supporting Information) indicated that drugs invaded the cells at the low chamber with the form of prodrug-assembling MBNs. Nevertheless, free DOX and DOX.HCl were very difficult to permeate into tumor cells at Transwell filter and low chamber (Figures S61 and S62, Supporting Information). These results suggested that MBNs were able to extravasate from vascular barriers and permeate into deeper tumor cells through cell-to-cell perforation. Comprehensive, developing virion-like MBNs is hopeful to extend a new tissue-penetrating model on the membrane-breaking perforation one after another.

Thereafter, multicellular tumor spheroids (MTSs) were built as 3D in vitro tumor models to evaluate tumor penetration, because MTSs are equipped with critical physiological parameters as in vivo tumor tissue, such as complicated multicellular architecture and multiple barriers to molecular transport. After treatment with MBNs at pH 7.4 culture medium for 4 h, only little red fluorescence was detected in the SKOV3/R MTS across each equatorial section in CLSM images (Figure 3B), and MTSs contained 0.2% DOX-positive cells by fluorescence-activated cell sorter (FACS) analysis (Figure S72, Supporting Information). On the contrary, within pH 6.5 culture media mimicking tumor microenvironment, red fluorescence fully distributed in each optical section, and the virus-like MBNs nearly infected most of SKOV3/R tumor cells (93.8%) in MTSs. Topological 3D view images visually reflected the total fluorescence intensity in the SKOV3/R MTSs. Much higher fluorescence intensity was observed in MTSs at pH 6.5, which was well agreed with MFI (Figure S73, Supporting Information). As compared with the tumor-penetrating ability of DOX.HCl (9.5% DOX-positive cells) and lysine-rich nanoparticles, much deeper penetration of arginine-rich MBNs in MTSs once again highlighted the importance of tailor-made molecular and supramolecular fabrication for efficient membrane-breaking tumor penetration.

To further investigate the tumor-penetrating ability of MBNs, SKOV3/R cells were uniformly seeded in 3D µ-slide chamber with matrigel, making the in vitro models much closer to in vivo tumor microenvironment (Figure 3C). After treatment with different formulations, 3D-reconstructed CLSM images intuitively presented their penetrating ability in the biomimetic tumor tissue. The activated MBNs (exposure to pH 6.5 microenvironment) vigorously penetrated into the deep section of biomimetic tumor tissue (more than 600 µm). These results indicate that MBNs could overcome the extracellular matrix and penetrate into tumor tissue because of its excellent membrane-breaking ability. In line with preceding results, free drugs (DOX and DOX.HCl) and unactivated MBNs only distributed in superficial layer of biomimetic tumor tissue (Figure S74, Supporting Information).

Following the analysis of penetrating ability by in vitro models, we turned to investigate in vivo drug distribution at tumor sites using nude mice bearing SKOV3/R xenograft tumors, which are notoriously difficult to permeate due to their low vascular density and poor extravasation. After intravenously injecting MBNs into nude mice for 24 h, solid tumors were excised for frozen sections. In the line with previous studies, native drug (DOX.HCl) and inert arginine-rich nanoparticles (I-MBNs) poorly distributed
in tumor tissue (Figure S75, Supporting Information; Figure 3D), limiting by inherent physiological barrier of SKOV3/R tumor. Encouragingly, extensive areas with strong fluorescence signals of MBNs were observed in the tumor cryosections, thanks to their robust membrane-breaking capacity in solid tumor. More importantly, the white-outlining region in Figure 3D showed that MBNs (red fluorescence) passed through endothelial cells and extravasated from vessel (FITC-CD31 staining green color), as well as deeply permeated into distant tumor cells. As a result, it was concluded that our virus-like MBNs can permeate into deep region at tumor tissue to improve therapeutic distribution and overcome chemotherapy resistance.

Once we confirmed the highly efficient cell-and-tumor dual-penetration of MBNs, in vitro antitumor activity to drug-resistant SKOV3/R cells was evaluated by CCK-8 assay. As shown in Figure 4A, the profiles of cell viability versus drug concentration manifested that MBNs had the highest antitumor activity to SKOV3/R cells at pH 6.5 culture media mimicking the tumor extracellular microenvironment, supporting the superiority of membrane-breaking cell penetration for defeating multidrug resistance. Notably, the acid-activated virus-like MBNs had quite a low IC50 value of 1.86 μg mL⁻¹ (the concentration causing 50% growth inhibition), which was reduced more than 30 times as compared with native DOX (61.62 μg mL⁻¹). And the IC50 value of MBNs was even lower than that of positive control (DOX·HCl, 7.50 μg mL⁻¹). However, after incubation with I-MBNs at pH 6.5 and MBNs at pH 7.4, their cytotoxicity to SKOV3/R cells tremendously reduced due to unactivated membrane-breaking ability (Figure S76, Supporting Information). In addition, we found that MBNs based on dendritic peptide prodrugs often provide much higher cytotoxicity against SKOV3/R tumor cells than that of nanoparticles assembled from Dend-R4K2K-Drug (amido linkage), especially with long incubation time (Figure S77, Supporting Information). These results supported that the design of pH-responsive linker within MBNs facilitated therapeutic effects following the drug release.

As with virus infection in vivo, the stealthy MBNs had little interactions with biomolecules (such as bovine serum albumin, Figure S80, Supporting Information) under normal physiological condition. However, once the virus-like MBNs recognized acid tumor microenvironment, the arginine-rich corona of MBNs would be activated for membrane-breaking penetration with significant interactions with proteins. The plasma drug concentration versus injection time profiles (Figure 4B) indicated that MBNs had a 10-fold longer half-life period (t1/2, 3.45 h) as compared with native DOX·HCl (0.33 h), and the area under the curve (AUC0–∞) of MBNs (679.5 mg L⁻¹ h) was about 13.8 times higher than DOX·HCl (49.4 mg L⁻¹ h). More pharmacokinetic parameters were listed in Table S3 (Supporting Information), and these results consistently confirmed

![Figure 4](https://www.advancedsciencenews.com/fig/1707240_f4.jpg)

**Figure 4.** In vitro and in vivo antitumor efficiency. A) Cytotoxicity of the DOX, DOX·HCl, MBNs, and I-MBNs toward SKOV3/R tumor cells with the different drug concentrations at pH 6.5 after 48 h incubation. B) Pharmacokinetic profiles after intravenous injection of DOX·HCl, MBNs, and I-MBNs in BALB/c mice at a drug dose of 10 mg kg⁻¹. C) Fluorescence images for SKOV3/R tumor-bearing nude mice after intravenous injection with DOX·HCl, MBNs, and I-MBNs (10 mg DOX·HCl⁻¹). D) Tumor volumes of SKOV3/R tumor-bearing nude mice after intravenous injection of normal saline, DOX·HCl, MBNs, and I-MBNs at a dose of 5 mg DOX·HCl⁻¹ over 21 d (n = 6; *p < 0.0001 by one-way analysis of variance, ANOVA). E) Histological and immunohistochemical images for H&E, Ki-67, and TUNEL assays of SKOV3/R tumor tissues after treatment with normal saline (control group), DOX·HCl, MBNs, and I-MBNs.

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the advantage of MBNs on prolonging the blood circulation, comparable to that of our previous PEGylated dendrimeric nanoassemblies.[1][74] In the following, we used a fluorescence imaging system for small animals to investigate in vivo drug distribution in nude mice bearing SKOV3/R tumors (Figure 4C). After intravenous injection for 1 h, drug fluorescence distinctly accumulated at tumor sites both in the MBNs and I-MBNs groups. As time goes on, stronger and stronger fluorescence signal of MBNs was enriched at solid tumor, because MBNs would recognize the tumor microenvironment to initiate tumor penetration and drug accumulation. However, I-MBNs had poor interaction with tumor tissue after arrival at tumor by enhanced permeability and retention (EPR) effects, thereby the fluorescence signal of I-MBNs at tumor site slightly increased at first and then decreased due to biological clearance. During the whole monitoring process, little fluorescence signal was detectable in the DOX.HCl-treated groups, because of rapid blood clearance and nonspecific tissue distribution. In keeping with in vivo imaging, the ex vivo imaging suggested MBNs accumulated and penetrated at solid tumor effectually.

The ultimate goal of efficient cell-and-tissue dual-penetration is to smoothly overcome intricate drug resistance during systemic chemotherapy. To evaluate in vivo antitumor efficacy of the virus-like MBNs, nude mice bearing SKOV3/R tumors were used as animal models following systemic administration. As recorded in Figure 4D, the tumor volumes of the normal saline group quickly increased to about 1200 mm³ after treatment course over 21 d (Figure S82, Supporting Information). The modest tumor inhibition was found in the DOX.HCl-treated mice as compared with control group of the normal saline-treated mice, due to the serious drug resistance of SKOV3/R xenograft tumors to antitumor drugs. However, the virus-like MBNs efficiently suppressed the growth of drug-resistant tumors, and the average tumor volume of MBN-treated mice was less than half the size of the DOX.HCl-treated mice. In the meantime, we found that the inert arginine-rich nanoparticles failed to inhibit drug-resistant solid tumor, further supporting that the cell-and-tumor dual-penetration based on acid-activated MBNs is vital for combating drug resistance. Accordingly, hematoxylin and eosin (H&E) staining of tumor slices also demonstrated the superabundant tumor cells in tumor tissue after administration with normal saline and I-MBNs (Figure 4E). Fortunately, MBNs caused the most damage and necrosis in tumor tissue, much more efficient than the DOX.HCl treatment. At the same time, the immunohistochemical studies manifested that few Ki-67-positive proliferating cells and many terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive apoptotic cells were clearly observed in the tumor tissue of mice treated with MBNs, unlike apparent proliferating cells and fewer apoptotic cells in other control groups. Moreover, treatment with MBNs also avoided the toxic side effects of DOX chemotherapy, having stable body weight and normal heart tissue (Figures S83 and S84, Supporting Information). Altogether, the virion-like MBNs with robust cell-and-tissue dual-penetration convincingly conquered the difficulty on chemotherapy of imperative multidrug-resistant cancer.

We have extended a new strategy on cell-and-tissue dual-penetration for defeating imperative drug-resistant tumor, relying on a bioinspired molecular and supramolecular construction of virion-like MBNs. These MBNs had well-organized secondary structure and nanostructure mimicking natural virions, and MBNs can recognize the tumor-specific pH microenvironment to arouse the arginine-rich corona as viral protein transduction domains. The activated MBNs could strongly break tumor membrane and invade tumor cells, immensely promoting chemotherapeutic efficacy to the drug-resistant cells. Most importantly, the robust virion-like MBNs can penetrate from the endothelial cells and tumor cells into other tumor cells, realizing transvascular extravasation and deep tumor invasion. In vitro and in vivo antitumor treatment indicated MBNs could surmount the imperative drug-resistant human ovarian cancer, attributing to the virus-mimicking nanostructures and functionalities, including stealth blood circulation to recognize the tumors and tumor-specific activation to initiate intense cell-and-tissue penetration. These MBNs are hopeful to defeat imperative solid tumors which often show low vascular density, small vessel diameter, and compact tumor interstitium. This work puts forward an unprecedented strategy on cell-and-tissue dual-penetration through the molecular and supramolecular development of virion-like membrane-breaking nanomimics to overcome the tough physiological barriers of imperative multidrug-resistant tumors.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

impermeable cancer, membrane-breaking capacity, multidrug resistance reversal, tumor penetration, virion-like nanoparticles

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