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Editorial

# Integrating targeting and drug retention: A promising approach for cancer treatment

Over the past decades, significant efforts have been devoted to developing drug delivery systems that specifically bind to tumor cells through distinct receptors. Prominent examples include antibody-drug conjugates [1] and peptide receptor radionuclide therapy [2]. Despite these advancements, receptor-mediated targeting is generally downplayed by inadequate tumoral drug retention as a result of the dynamic binding process that allows dissociation and fast clearance of therapeutics from cancer tissues. Interesting work, recently published by Liu and colleagues in *Nature*, reveals that 'covalent fixation' of targeted radioligands upon binding to cancer cells improves tumor accumulation of drugs significantly [3]. Given the tremendous medical need for a better targeting of compounds in tumors, in this Editorial we like to highlight some recent strategies for enhancing drug retention in tumors (Fig. 1).

Inspired by supramolecular assemblies as formed among proteins or lipids in biological systems, drug conjugates that form large assemblies *inside* living tumor cells in response to exogenous or endogenous stimuli (like an acidic pH, enzymes, reactive oxygen species), have been designed to prolong local drug concentration (Fig. 1A) [4,5]. Zhan et al. developed a 10-hydroxycamptothecin-peptide conjugate that, when triggered by alkaline phosphatase, self-assembles into nanovesicles extracellularly. These nanovesicles then transform into filaments intracellularly in response to the high levels of glutathione present in tumor cells. Compared to free drug, this morphological change significantly enhanced the intra-tumoral accumulation of the drug in xenograft models [6]. Different from enzyme-induced self-assembly of drug-peptide conjugates, Wang's group demonstrated the self-assembly of drug-peptide conjugates through covalent cross-linking, as exemplified by peptide-porphyrin conjugates containing thiols. These thiols undergo cross-linking to form dimers, which then organize and assemble into an artificial shell surrounding the oxidative mitochondria. Thus enhanced retention of the therapeutic agents resulted in a stronger antitumor activity [7]. Cao et al. designed doxorubicin-loaded nanoparticles composed of a peptide that includes an RGD sequence for tumor targeting and triphenylphosphine (TPP) as lipophilic targeting moiety for mitochondria. Triggered by extracellular alkaline phosphatase, which phosphorylates tyrosine residues in the peptide, the nanoparticles could be transformed into nanofibers with concomitant release of loaded doxorubicin. Subsequently a phase transition from nanofibers into a hydrogel, stimulated by intracellular glutathione, further enables intracellular retention of doxorubicin to sustainably induce immunogenic cell death [8]. Gu and colleagues reported a method for the *in situ* formation of biomolecular 'condensates' in cells, as published in *Nature Biomedical Engineering*. These condensates were sufficiently large to remain within tumor cells and served as reservoirs for the enrichment and retention of chemotherapeutics (doxorubicin) in cancer cells [9]. Intracellular *in situ* self-assembly of drug conjugates represent an appealing strategy to extend drug retention in tumor cells, however major challenges remain to translate it into clinical drug delivery systems. One concern is the chemical stability of the precursor molecules as peptide-drug conjugates might easily undergo proteolysis during circulation. Another concern is the high dose of peptide-drug conjugates required for self-assembly which might be too toxic. The fate of the *in situ*-formed nanostructures must also be considered, as stable assemblies might cause long-term adverse effects.

Aggregation of drug delivery systems, either in tumor blood vessels, on the membranes of cancer cells or in the extracellular space has been reported as well to enhance drug retention (Fig. 1B) [10]. As reported in *Nature Communications*, Cao and colleagues designed drug loaded nanoparticles that enlarge after extravasation into the extracellular space of the tumor, thereby retaining the nanoparticles within the tumor for an extended period and functioning as a local drug depot. After extravasating into the tumor site, the slightly acidic tumor environment induces the exposure of cysteine molecules on the surface of a nanoparticle, which subsequently reacts with 2-cyanobenzothiazole moieties on a neighboring nanoparticle, enabling the formation of micro-sized drug depots [11]. Wen et al. anchored drug-loaded nanoparticles onto tumor cell membranes *via* a cascade of alkaline phosphatase-mediated *in situ* self-assembly combined with a bioorthogonal inverse electron demand Diels-Alder reaction, effectively enriching therapeutic molecules (photosensitizers and carbonic anhydrase inhibitors) in tumors [12]. Wang et al. presented peptide-drug conjugates that target carbonic anhydrase IX on the surface of cancer stem-like cells and self-assemble into nanofibers through surface-induced *in situ* self-assembly, improving drug retention at tumor sites [13]. The *in situ* self-assembly approach utilizing a stimuli-responsive small-molecule cisplatin prodrug activated by extracellular alkaline phosphatase effectively extends drug retention and improves endocytosis into tumor cells [14]. In addition to chemotherapeutics, Cai's group introduced a peptide-antibody combo-supramolecular *in situ* assembled CD47 and CD24 bi-target inhibitor, which undergoes biomimetic surface propagation on tumor cell membranes through ligand-receptor interactions and enzyme-triggered reactions [15]. The formation of superstructures facilitates their effective accumulation and retention in tumors for up to 120 h, potentially providing sustained blockade of innate immune checkpoints and thereby inducing prolonged macrophage-mediated immune responses. As one can read in Wu et al., targeting the extracellular matrix (ECM) has been proposed as a strategy to prolong drug accumulation in cancer tissues. To this end, bioinspired lipoproteins are engineered to preferentially bind to ECM compounds, such as tenascin C

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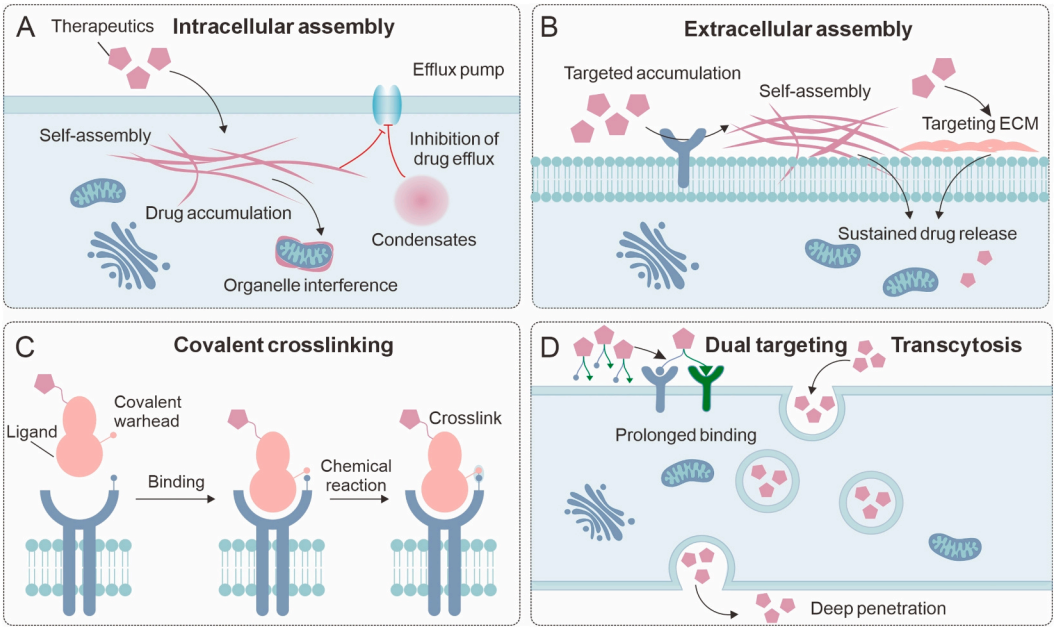


**Fig. 1.** Emerging approaches to enhance tumoral drug retention in targeted cancer therapy. **(A)** The intracellular self-assembly of drug conjugates (indicated as 'therapeutics'), triggered by either endogenous or exogenous stimuli, has been investigated to increase local drug concentration and extend drug exposure by mitigating drug efflux. **(B)** Extracellular aggregation and self-assembly of drug delivery systems, along with the targeting of the extracellular matrix, might present significant potential for enabling sustained drug release. **(C)** The integration of targeted binding with covalent crosslinking represents a robust approach to improve the attachment of therapeutics to tumor cell membranes, thereby reducing the rapid clearance of these agents by tumors. **(D)** Dual-targeting strategies and transcytosis mechanisms are effective methods for promoting the extravasation and retention of therapeutics within tumor tissues.

and/or fibronectin, thereby enhancing the retention of cabazitaxel and pomalidomide in tumors [16]. Extracellular aggregation of drug delivery systems and self-assembly of drug-conjugates in the ECM might be promising strategies, however, condensed aggregates or assemblies in the interstitial space might hinder the infiltration of cytotoxic immune cells. Moreover, the extracellular release of anticancer drugs might lead to poor cell selectivity.

'Covalent drugs' feature a mildly reactive functional group that establishes a covalent bond with protein targets, thus enhancing affinity beyond the non-covalent interactions typically involved in binding drugs to receptors [17]. 'Covalent cross-linking of drugs' to tumor cells (Fig. 1 C) has become a promising approach to extend the duration of action of drugs. Notably, Wang's group introduced 'covalent proteins' for targeting radionuclides by genetically engineering a latent bioreactive amino acid into a nanobody [18]. This modification enables the nanobody to bind its receptor while establishing a covalent bond through proximity-driven reactivity, significantly elevating the accumulation and prolonging the residence time of the radioisotope in the tumor. Liu's team utilized a sulfur (VI) fluoride exchange (SuFEx) chemistry-based linker on radiopharmaceuticals to mitigate the rapid clearance from tumors [3]. Upon reaching the tumor, the targeting ligand initially binds noncovalently to the tumor-specific fibroblast activation protein (FAP) before undergoing a binding-to-ligation transition, which facilitates conjugation to tyrosine residues *via* the 'click' SuFEx reaction. An impressive 80 % of covalent targeted radioligand remained covalently bound to the target protein with negligible dissociation over a span of 6 days.

Bioorthogonal chemistry refers to the selective reactions between abiotic functional groups that do not interact with biological functionalities, demonstrating considerable promise for tumor-specific targeting and enhanced drug retention [19]. Ding's group pre-modified tumor cells with tetrazine groups, effectively converting the tumor into a "drug factory" capable of activating an externally administered bioorthogonal prodrug, such as transcyclooctene-caged doxorubicin, *in situ* [20]. Unlike the rapid clearance of cargo encapsulated and delivered by liposomes, this bioorthogonal approach facilitates a sustained release of doxorubicin within the tumor environment for up to 96 h. Covalent binding serves as an effective strategy to mitigate the rapid clearance of tumors. Given the wide variety of active functional groups present on cell membranes, numerous crosslinking reactions warrant further investigation. Notably, as the active groups on the cell membrane are not exclusive, non-specific cross-linking during circulation could result in binding to normal cells.

In addition to self-assembly and chemical coupling, innovative strategies such as dual-targeting and transcytosis have been investigated to enhance drug retention (Fig. 1D). Dual and multiple targeting ligands exhibit promising characteristics, including enhanced tumor uptake, improved tumor-to-normal tissue ratios, and extended tumor retention times. Recently, we published a study in *Journal of Controlled Release* describing a dual-targeting strategy that utilizes a peptide to simultaneously target the Sigma-1 receptor and prostate-specific membrane antigen (PSMA), thereby enhancing peptide receptor radionuclide therapy of prostate cancers. In comparison to a PSMA single-targeted control, our dual-targeting method results in approximately 2-fold greater tumor accumulation, while significantly diminishing uptake in most normal tissues [21].

Transcytosis is an active mechanism through which macromolecular cargoes are first internalized from the apical surface and subsequently exocytosed from the basal side *via* intracellular transport. This process has been harnessed to enhance drug extravasation and penetration into tumors [22]. Shen's research group has performed comprehensive studies on active transcytosis-mediated nanomedicine. Recently, they evaluated a series of polyglutamine-paclitaxel conjugates, with varying hydrophilic/hydrophobic ratios and proportions of tertiary amine-oxide, thereby selectively targeting the Golgi apparatus to promote transcytosis and facilitate accumulation and penetration within tumor tissues [23]. Su et al. developed a liposomal system modified with protamine, enabling transcytosis across endothelial cells while maintaining structural integrity. This modification supports subsequent drug retention and release within tumor cells through degradation-induced aggregation [24].

Although both dual-targeting and transcytosis strategies have been

demonstrated to greatly improve extravasation and retention of drugs within tumors, several considerations should be taken into account. In the dual-targeting approach, careful design is required to allow simultaneous binding to two receptors, which is challenged by spatial arrangements. For transcytosis, drug conjugates or nanovesicles might require sophisticated synthetic processes, which could pose obstacles for clinical translation.

The accumulation of drugs within tumor tissues constitutes a critical factor in evaluating the efficacy of systemically administered therapeutics for solid tumors. Significant efforts have been made to enhance the targeting efficiency of these treatments; however, inadequate retention in tumor sites leads to limited drug uptake and may facilitate increased distribution to healthy tissues, thereby exacerbating off-target toxicity. Consequently, it is essential to reassess the importance of improving tumor retention in the context of targeted tumor therapies. Tumor targeting serves as the initial phase, succeeded by the stabilization of drugs within the tumor through various innovative strategies such as *in situ* self-assembly, stimuli-responsive aggregation, chemical crosslinking, dual or multiple targeting, and transcytosis. The exploration of novel methodologies aimed at extending drug retention represents a burgeoning area within targeted drug delivery.

In the future, prolonged drug retention initiated *via* the single or combinatorial use of extracellular or intracellular tumor-specific environments and endogenous or exogenous stimuli should be pursued to achieve high efficacy and safety. In the meantime, the eventual clearance of foreign materials should be ensured. Imitation of nature, by utilizing or mimicking natural retention behaviors, could be advantageous. For example, exploring naturally occurring abiogenetic transcytosis might provide innovative insights for intratumoral drug penetration and retention. In addition to traditional cytotoxic agents like chemotherapeutics and radiotherapeutics, prolonged retention of new therapeutic modalities, such as immunoadjuvants, cytokines and nucleic acids, might significantly improve the therapeutic outcomes by reversing the suppressive immune microenvironment. As advancements continue, integrating targeting and retention strategies might become a leading clinical approach in cancer treatment.

## Declaration of competing interest

None.

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