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Review article

# Chemotherapeutic drug delivery by tumoral extracellular matrix targeting

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ABSTRACT

Systemic chemotherapy is a primary strategy in the treatment of cancer, but comes with a number of limitations such as toxicity and unfavorable biodistribution. To overcome these issues, numerous targeting systems for specific delivery of chemotherapeutics to tumor cells have been designed and evaluated. Such strategies generally address subsets of tumor cells, still allowing the progressive growth of tumor cells not expressing the target. Moreover, tumor stem cells and tumor supportive cells, such as cancer associated fibroblasts and cancer associated macrophages, are left unaffected by this approach. In this review, we discuss an alternative targeting strategy aimed at delivery of anti-tumor drugs to the tumoral extracellular matrix with the potential to eliminate all cell types. The extracellular matrix of tumors is vastly different from that of healthy tissue and offers hooks for targeted drug delivery. It is concluded that matrix targeting is promising, but that clinical studies are required to evaluate translation.

## 1. Introduction

Targeted drug delivery of chemotherapeutics is an increasingly important area in the field of cancer treatment research. Although conventional chemotherapy remains one of the most important treatment modalities, significant side effects may be induced that can result in preliminary cease of chemotherapy [1–3]. Moreover, chemotherapeutics have an unfavorable biodistribution and are generally rapidly removed from the body. Advanced drug delivery systems may overcome these hurdles. By entrapping chemotherapeutics in a drug delivery system, exposure to healthy cells may be decreased, which, together with an increased concentration of chemotherapeutics specifically at the tumor site, can result in enhanced treatment efficacy with reduced side effects [4]. However, to achieve this, drug delivery systems should be designed to deliver chemotherapeutics to tumors only and not to surrounding healthy tissue. Several approaches, including passive targeting and ligand mediated targeting, are currently being evaluated to achieve local delivery to tumor cells. While the majority of the field is focusing on targeting the tumor cells itself, we here discuss an alternative approach *i.e.* targeting the tumor's extracellular matrix (ECM). This strategy may result in a higher treatment efficacy by affecting not only tumor cells, but also tumor supportive cells. Tumor supportive cells are considered to have major roles in supporting tumor growth. The cancer associated fibroblast (CAF), for instance, is a tumor-distinctive cell type responsible for excretion of proliferating, pro-angiogenic, and anti-immunogenic factors, creating an ideal environment for tumor growth and subsequent metastasis [5]. Further, the cancer associated macrophage shares many of the tumor supportive characteristics of CAFs. Once derived from monocytes to its specific subtype and located in the ECM of tumors the cancer associated macrophage is thought to produce and secrete tumor enhancing factors [6,7]. Endothelial cells are another cell type considered as key players in tumor growth. By facilitating the supply of nutrients (*e.g.* oxygen, glucose, *etc.*) through the generation and support of novel blood vessels, tumors continue to proliferate [8]. Finally, the tumor stem cell is a major player in tumor progression. Tumor stem cells are considered responsible for self-renewal of tumor cells thereby driving tumor growth [9,10]. A strategy that simultaneously affects tumor cells and tumor supportive cells may be beneficial in improving treatment efficacies. We will present an overview of the possibilities and limitations of strategies to deliver chemotherapeutics to and release them in the tumor extracellular matrix.

## 2. Conventional tumor targeting strategies

Passive targeting is one of the main strategies to guide drug delivery systems to cancer cells making use of the enhanced permeability and retention effect (EPR) [11]. This phenomenon is based on newly formed leaky vessels in tumor areas (permeability) with decreased lymphatic drainage resulting in an increased retention [12]. As a result, accumulation of drug delivery systems at the tumor site may occur. Despite extensive evaluation over the last 30 years and initial promising

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preclinical results of passively targeted chemotherapeutic drug delivery through the EPR effect, serious questions have been raised about the existence and clinical application of the EPR effect in humans [13,14].

The opposite of passive targeting, active targeting, is therefore under growing attention. Active targeting is based on the concept that drug delivery systems can actively bind and subsequently internalize into tumor cells using tumor cell specific antibodies or ligands [11,15]. The ideal target is highly overexpressed on tumor cells, and absent or expressed to a limited extent on healthy cells. Examples are membrane bound receptors such as the human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor-1 (EGFR), transferrin receptor and folate receptor-α. However, there are several limitations associated with targeted delivery to tumors that need to be resolved in order to further improve the treatment outcome.

## 3. Limitations of current targeting strategies

Conventional drug delivery systems that target tumor cells through binding membrane bound molecules have several pitfalls. First, tumors show a high intratumoral heterogeneity resulting in heterogeneous expression of targets for drug delivery [16]. This may implicate that in practice targeted therapy may result in removal of only the subset of tumor cells expressing the target, while tumor cells lacking the target are left unaffected. As a result, these cells may proceed proliferating and finally result in a tumor lacking expression of the initial target (Fig. 1).

A second aspect of the current tumor targeting is the disregard of tumor supportive cells being present in the tumoral extracellular matrix (ECM). Tumor supportive cells are responsible for important cues for tumor proliferation and involved in the maintenance of a tumor supportive environment. Drug delivery strategies aimed at a specific tumor cell can leave other tumor cells and, perhaps even more importantly, tumor stem cells, tumor supportive cells and their supportive environment intact and may therefore not be sufficient to eradicate the whole tumor and prevent relapse.

Thirdly, delivery of entrapped drugs to their location of action by a targeted drug delivery system has proven more complex than initially anticipated. The majority of the current targeting drug delivery systems are designed to deliver their payload to their site of action (*e.g.* the nucleus). For most chemotherapeutics, this implicates that once a drug delivery system is bound to its target (*e.g.* membrane receptor) rapid internalization should occur. Thereafter, the drug should be released and subsequently move to its site of action, and not diffuse back into circulation. Although targeting the tumor cell membrane with subsequent internalization can be accomplished using antibodies or ligands, the steps to deliver chemotherapeutics to its site of action are more complicated. Once internalized into lysosomal compartments in the cytoplasm, the drug should be released from its carrier. A wide range of drug delivery systems struggle to release their payload after internalization because of failure to escape from lysosomal compartments in which they end up after internalization [17,18]. For example, the majority of injected PEGylated liposomal doxorubicin, a clinically approved passive targeting drug delivery system for doxorubicin, is found to be entrapped in lysosomes after internalization. *In vivo* experiments have shown that as a consequence of this lysosomal entrapment, less than 1% of the administered doxorubicin from liposomes reaches the nucleus, its actual target [19]. Lysosomal sequestering of drug delivery systems following internalization can thus prevent chemotherapeutics from reaching their site of action.

Overall, the majority of the tumor targeting drug delivery strategies for chemotherapeutic delivery focus on targeting tumor cells and may result in eradication of only a specific subset of tumor cells. Importantly, tumor supportive cells and tumor stem cells are left unaffected. Impaired release of chemotherapeutics and lysosomal entrapment may further limit the treatment efficacy. Therefore, an alternative targeting strategy that tackles these issues is desired.

## 4. Alternative tumor targeting strategy

Targeting chemotherapeutics to the extracellular matrix (ECM) of tumors may be a promising alternative strategy that can offer advantages over conventional targeting. The strategy is not aimed to target membrane bound receptors on specific tumor cells, but is aimed to target the unique tumoral ECM. Upon binding, depots of chemotherapeutic carriers in the tumor ECM are formed. Finally, when chemotherapeutics are released in the ECM they are able to diffuse to and affect all surrounding tumor cells including the heterogeneous tumor cell subsets, tumor supportive cells and tumor stem cells (Fig. 2). Here we will discuss this emerging field and define a number of conditions necessary to use drug delivery systems or antibody-drug conjugates as local extracellular chemotherapeutic depots.

### 4.1. The unique tumoral extracellular matrix

The normal ECM has many important functions including support

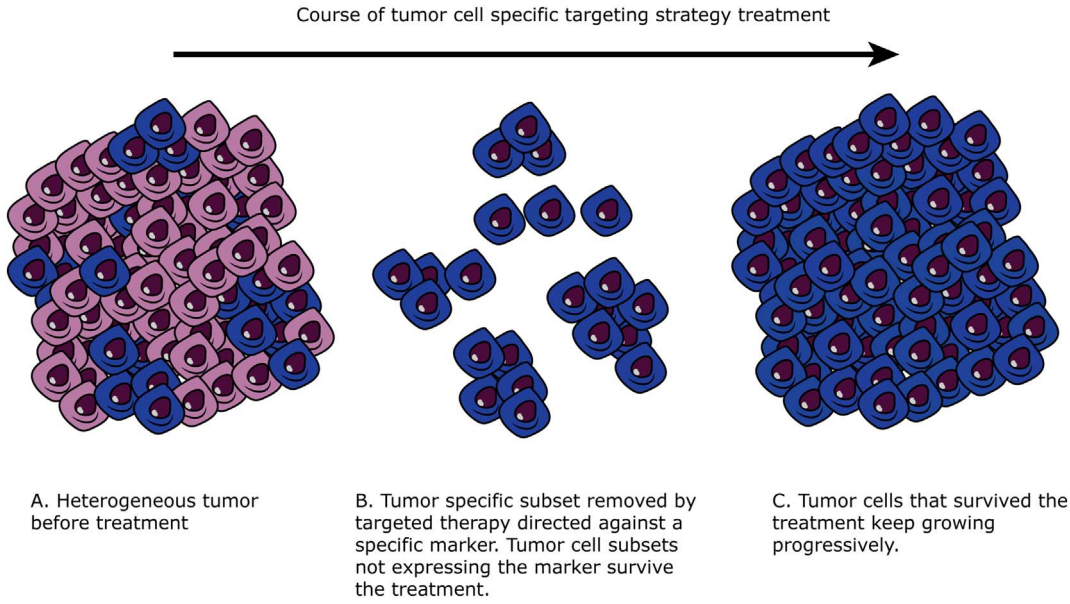


**Fig. 1.** Limitations of the conventional tumor targeting strategy. By addressing a specific tumor marker, tumor cells lacking the marker may survive and continue to grow progressively.

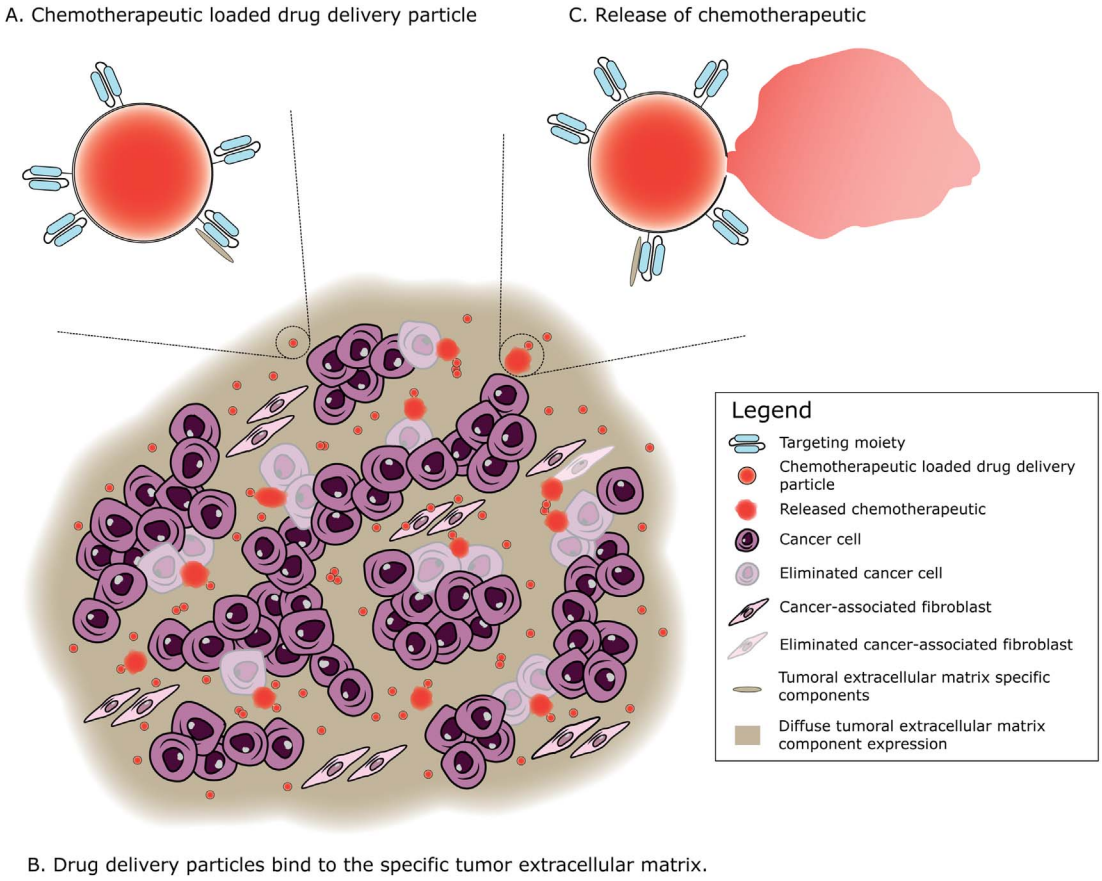


**Fig. 2.** A schematic overview of targeting to the extracellular matrix. Drug delivery systems bind to a molecule abundantly present in the extracellular matrix of tumors. Upon binding, the chemotherapeutic compound is released and diffuses into all tumor cell subsets, but also into other cells (*e.g.* cancer associated fibroblasts, cancer associated macrophages, tumor stem cells). Adapted from Van der Steen et al. [20] under the Creative Commons Attribution License (CC BY 4.0).

and strength for tissues. It consists of various components such as (glyco)proteins (*e.g.* tenascin, collagen, elastin, laminin, fibronectin, and proteoglycans) and glycosaminoglycans (*e.g.* heparan sulfate and chondroitin sulfate) [21,22]. The tumoral ECM is considered distinct from normal tissue ECM in various aspects. For instance, several types of collagen are abundantly deposited during tumor formation and chondroitin sulfate and heparan sulfate are also more abundantly present in the ECM of tumors, both having the capacity to bind tumor promoting growth factors. Moreover, ECM remodeling enzymes are overexpressed in tumors [23]. It is believed that these factors contribute to tumor progression and invasion. Consequently, the deregulated tumoral ECM may also provide targeting possibilities as the tumor ECM may be enriched in certain molecules that are almost absent in normal ECM.

### 4.2. Targets in the tumoral extracellular matrix

As mentioned, the tumoral ECM is distinct from the normal ECM and may offer targeting possibilities. To ensure specific tumor delivery, the target should be expressed specifically in the ECM of tumors. Several ECM targets that may be used for drug delivery have been described as suitable. An overview of tumor ECM targeting strategies is presented in Table 1, and some examples will be discussed here.

Tenascin-C is a large glycoprotein of about 300 kDa which is highly expressed in the ECM of several tumors including breast, colon, lung, and ovarian tumors. It supports several aspects of tumor growth, such as tumor proliferation, angiogenesis and metastasis [24]. Moreover, its expression in normal ECM is almost absent [24], making it suitable for ECM targeting. Dal Corso et al. used a non-internalizing antibody directed against tenascin-C to deliver a chemotherapeutic compound (the anthracycline PNU159682) to the ECM of tumors [25]. Upon intravenous injection, the antibody-drug conjugate bound to tenascin-C (Fig. 3) and the drug was released through cleavage of the protease-sensitive linker between the drug and antibody. Significant tumor growth inhibition was observed in epidermoid carcinoma mouse xenografts. Chen et al. developed a strategy targeting tenascin-C using liposomes functionalized with a tenascin-C binding peptide and loaded with navitoclax, a small molecule inducing apoptosis primarily in CAFs. These liposomes modulated the ECM of tumors through efficient removal of CAFs, making the ECM more accessible for subsequently administered doxorubicin loaded nanoparticles [26,27]. Because only CAFs were affected by the initial ECM targeting strategy, they still had to apply a subsequent tumor cell specific targeting method. Using nanoparticles containing doxorubicin and targeting the human transferrin receptor, significant tumor growth inhibition was observed in liver tumor-bearing mice. Kang et al. targeted both, tumor cells using neuropilin-1 and tumor ECM tenascin-C with nanoparticles for glioma therapy. When loaded with paclitaxel, they tripled the median survival of intracranial glioma tumor bearing mice [28]. Lin et al. evaluated another tenascin-C targeting strategy. Doxorubicin loaded liposomes functionalized with sulfatide, a tenascin-C binding glycosphingolipid, were evaluated in mice bearing subcutaneous colorectal tumors and subcutaneous glioma tumors [29–32]. Although prolonged survival and decreased side effects were observed, the strategy was still dependent on endocytic cellular uptake of liposomes by glioma cells, which may limit the full potential of this strategy due to lysosomal entrapment of the liposomes. Another tenascin-C targeting approach was evaluated by Li et al. in a breast cancer mouse model. Mice were treated with paclitaxel loaded sulfatide-containing lipid nanoparticles. Again, despite increased efficacy over non-targeted delivery and free drug, the

**Table 1**
Overview of drug delivery strategies targeting the extracellular matrix in tumors.

| Target | Targeting system | Payload | Remarks | References |
|---|---|---|---|---|
| Tenascin-C | Antibody drug conjugate | Anthracycline PNU159682 | ECM release | [25] |
| | FHKHKSPALSPVGGG peptide-liposomes[a] | Navitoclax | Removal of CAFs, subsequent tumor cell targeting of liposomes required | [26,27] |
| | FHKHKSPALSPV peptide- tLyp-1-peptide nanoparticles[a] | Paclitaxel | | [28] |
| | Sulfatide-liposomes[a] | Doxorubicin | | [29–32] |
| | Sulfatide-nanoparticles[a] | Paclitaxel | | [33] |
| Fibronectin extra domain A or B | Antibody (SIP-F8) drug conjugate (extra domain A) | Maytansinoid derivative MD1 | Extracellular release strategy | [38] |
| | Aptide-nanoparticles (extra domain B) | Iron oxide | Imaging only | [39] |
| | Single chain variable fragment (CGS-1)-liposomes[a] (extra domain B) | 5-FdU-NOAC | | [40] |
| | Single chain variable fragment (L19)-interleukin 2 fusion protein (extra domain B) | Interleukin 2 | Stimulation of immune response | [41] |
| Fibronectin-fibrin complex | CLT-1 peptide-FITC conjugation | FITC | Imaging only | [42] |
| | CLT-1 peptide-nanoparticles[a] | Paclitaxel | | [43] |
| | CLT-1 imaging complex | Gadolinium | Imaging only | [44,45] |
| | CREKA-nanoparticles | Iron oxide | *In vitro* targeting | [46] |
| | CREKA-thermosensitive-liposomes | Doxorubicin | ECM release by external heat | [47] |
| | Antibody-drug conjugate | SN-38 | ECM release | [48] |
| Collagen | Antibody-drug conjugate to type IV collagen | SN-38 | ECM release | [49] |
| | Collagen-binding domain peptide fused with Fab fragment of an antibody against EGFR (type of collagen not specified) | None | Membrane receptor binding of specific tumor cell subset required | [50] |
| Galectin-1 | Anginex galectin-1 binding peptide-liposomes[a] | Cisplatin and arsenic trioxide | | [51,52] |
| Aggrecan | Quaternary ammonium-drug-conjugate | Melphalan | Therapeutic mechanism not clear | [53–55] |
| Heparan sulfate | CGKRK peptide nanoparticles | Paclitaxel | Dual targeting to heparan sulfate and endothelial cells | [57] |
| Chondroitin sulfate | TRX-20 modified liposomes[a] | Cisplatin | | [59] |
| | Single chain variable fragment (GD3G7)-lyophilisomes (against CS type E) | Doxorubicin | ECM release, *in vitro* study | [20] |

[a] Internalization of carrier + drug required for therapeutic activity, endosomal escape necessary.

nanoparticles had to internalize into tumor cells in order to function [33]. It should be noted, however, that sulfatide, while suitable for incorporation in lipid based drug delivery particles (*e.g.* liposomes) and favoring binding to tenascin-C, has been reported to bind other matrix molecules [32], which could result in potential off-target effects. Therefore, the use of a tenascin-C binding peptide or a selected aptamer [34,35] may be preferred. Overall, tenascin-C appears to be a promising target because of its almost exclusive expression in the ECM of tumors. However, most tenascin-C targeting strategies could be improved by introducing drug release in the ECM to overcome potential lysosomal sequestration of the internalized drug delivery particles.

Another molecule to target in the ECM of tumors is fibronectin, a glycoprotein consisting of two 250 kDa subunits. Two alternatively spliced isoforms (*i.e.* extra domain-A and -B) of fibronectin are abundantly present in the tumor ECM of various types of cancer and almost absent in the normal ECM, making them prospective targets for ECM drug delivery (as extensively reviewed in [36,37]). A few studies evaluated fibronectin as a target for ECM targeted cancer treatment. For instance, Perrino et al. showed that a straightforward strategy, targeting the fibronectin extra domain-A with an antibody-drug (maytansinoid derivative MD1) conjugate, was able to induce complete remission in a subcutaneous teratocarcinoma mouse model [38]. Interestingly, this approach was based on non-internalizing antibodies that released the drug extracellularly due to the release of reducing agents (*e.g.* cysteine or glutathione) from dying cells which then released the drug by reduction of the disulfide bonds. These results suggest that release of a chemotherapeutic drug in the ECM of tumors can indeed affect the whole tumor. Additionally, it was shown by Park et al. that nanoparticles functionalized with peptides with a high affinity towards fibronectin extra domain B accumulated specifically in tumors of a Lewis lung carcinoma model [39]. Alternatively, other groups used a combination of liposomes with single chain variable fragment (scFv) antibodies against fibronectin extra domain B for ECM targeting. However, therapeutic *in vivo* studies in mice bearing subcutaneous teratocarcinomas treated with fibronectin extra domain B targeting liposomes loaded with the cytotoxic compound 5-FdU-NOAC showed no significant differences in comparison to non-targeting liposomes [40]. This lack of therapeutic advantage may be due to the absence of an extracellular release mechanism resulting in the required internalization of liposomes for therapeutic activity. Carnemolla et al. were able to successfully target the fibronectin extra domain B using a biologically active fusion protein of interleukin-2 and a scFv antibody (clone L19) to induce an anti-tumor immune response [41]. Subcutaneous F9-mouse teratocarcinoma tumors were significantly smaller when treated with the interleukin-2-scFv conjugate, indicating that the L19 scFv may be a suitable candidate to use for tumoral ECM drug delivery.

Others have targeted fibronectin in the tumor ECM through its interaction with plasma proteins. Plasma proteins extravasating through vessels can form complexes with fibronectin such as fibrin-fibronectin [42]. This tumor specific complex, also called clotted plasma proteins, may be used as a target to deliver chemotherapeutics to the tumor ECM. In a glioblastoma mouse model, nanoparticles functionalized with CLT-1 (CGLIIQKNEC), a fibrin-fibronectin binding peptide, loaded with paclitaxel, were able to significantly prolong survival. However, a possible by-stander effect by eliminating surrounding tumor (supportive) cells may have been limited because the strategy was dependent on integrin mediated cellular internalization into tumor cells and not on extracellular drug release [43]. Fibrin-fibronectin targeting with the CLT-1 peptide was also used by Tan et al. to visualize prostate tumors by MRI indicating the suitability of CLT-1 for tumor ECM targeting

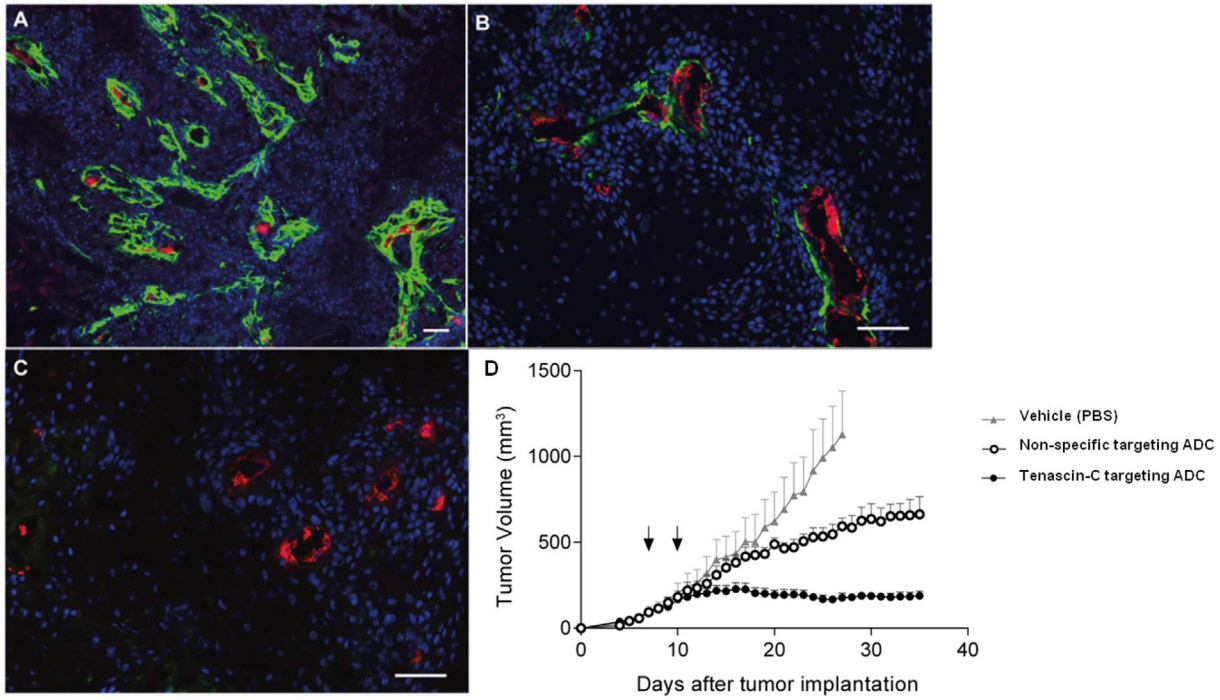


**Fig. 3.** An example of an *in vivo* tumoral extracellular matrix targeting strategy. (A) Mice bearing A431 human epidermoid carcinoma xenografts showed strong tenascin-C expression (green), especially around tumor vessels (red). (B) In tumors of mice treated with an antibody-drug conjugate directed against tenascin-C (green), the antibody was found to localize to tenascin-C in the direct area of tumor vessels (red). (C) Tumors of mice injected with a control antibody (directed against hen egg lysozyme) did not show presence of the antibody. (D) Results of an efficacy study in mice with A431 human epidermoid carcinomas that were treated with tenascin-C targeting antibody-drug conjugate (closed circles) indicate a statistically significant tumor growth inhibition compared to control antibody-drug conjugate (against hen egg lysozyme; open circles) or vehicle only (PBS; triangles). Data points represent mean tumor volume ± SEM, n = 5 per group. Blue: nuclei. Scale bars: 100 μm. Reprinted with permission [25]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[44,45]. Next, Kruse and co-workers used CREKA, a peptide that also binds fibrin-fibronectin complexes [46]. Although the iron oxide nanoparticles were able to specifically target fibrin-fibronectin *in vitro*, no *in vivo* tumor targeting or therapeutic studies were reported. CREKA was also used for tumoral ECM targeting by others. Wang et al. prepared doxorubicin loaded CREKA-functionalized liposomes with thermosensitive release characteristics. Mice bearing subcutaneous multidrug resistance adenocarcinomas showed significant inhibition in tumor growth when treated with doxorubicin loaded CREKA liposomes [47]. Moreover, when tumors were heated to induce doxorubicin release, tumor growth was even more inhibited, indicating that upon binding in the ECM of tumors, the released doxorubicin was able to reach its site-of-action and affected tumor growth. In a slightly different approach, Yasunage et al. targeted fibrin clots in the tumoral ECM using an antibody that was conjugated with the cytotoxic compound SN-38, the active metabolite of irinotecan, that was modified to be only cytotoxic upon release from the antibody due to an alkaline labile ester bond [48]. An *in vivo* therapeutic study in a chemically induced skin carcinoma mouse model showed a significant tumor growth inhibition in mice treated with the antibody-drug conjugate. Further analyses showed a significantly higher concentration of the drug in tumors of mice treated with the antibody drug conjugate, indicating that fibrin-fibronectin targeting in the tumor ECM may be useful for targeted chemotherapeutic drug delivery.

Although targeted less frequently, collagen may also be a potential target in the ECM of tumors. Collagen is a structural protein abundantly present in the ECM of most tissues. Despite the presence of collagen throughout the body and risk of off-targeting with toxicity as a result, several attempts have been made to target collagen in the tumoral ECM for the delivery of chemotherapeutics. Yasunaga et al. developed an antibody drug conjugate against type IV collagen that released the antineoplastic drug SN-38 through the labile ester bond linker in the tumor ECM [49]. Evaluation in mice with two types of subcutaneous pancreatic tumors (stroma poor and stroma rich tumors) showed almost complete tumor growth inhibition of stroma rich tumors treated with the anti-collagen drug conjugate. Interestingly, growth of stroma poor tumors was less affected by the antibody-drug conjugate suggesting specific targeting of stroma rich tumors. In spite of the abundant expression of collagen in the body, body weight was not affected and no toxicity in the liver, kidney and bone-marrow was observed, suggesting that distribution to other organs may be limited. Liang et al. also targeted collagen in the ECM of tumors. They designed an antibody drug conjugate by combining a collagen (collagen type not specified) binding domain peptide with the Fab fragment of a clinically approved antibody (cetuximab) directed against the epidermal growth factor receptor (EGFR) which has antitumor activity itself [50]. In a therapeutic *in vivo* study with mice bearing subcutaneous EGFR positive tumors, tumor growth was significantly inhibited in mice treated with the collagen binding domain-anti EGFR conjugate compared to cetuximab only. In general, the full potential of tumoral ECM targeting was not utilized because the therapeutic molecule required binding of a specific receptor (EGFR) on a tumor cell subset.

Next to collagen, galectin-1 has been used for targeting to the tumoral ECM. Galectin-1 is a carbohydrate binding protein that plays a role in cellular interactions. Underlining the limitations of cellular targeted therapies due to the tumor heterogeneity of triple negative breast cancer (*i.e.* breast tumors not overexpressing the estrogen receptor, progesterone receptor and epidermal growth factor receptor-2), Upreti and colleagues developed a tumor ECM targeting strategy directed at galectin-1 [51,52]. Cisplatin and arsenic trioxide loaded liposomes functionalized with anginex, a small galectin-1 binding peptide, were evaluated for their therapeutic efficacy in an orthotopic triple negative breast cancer mouse model. They showed significant tumor growth reduction compared to treatment with non-targeting drug loaded liposomes. Although initial results were promising, an increased efficacy may be reached by applying extracellular release of the

cytotoxic agents instead of using liposomes requiring receptor mediated endocytosis and release inside cells to be therapeutically active.

As proteoglycans are abundantly present in the ECM, they may be used for targeting chemotherapeutics to the tumor ECM as well. For instance, aggrecan, a proteoglycan expressed in the ECM of cartilage but also abundantly present in the ECM of chondrosacromas was targeted by Peyrode et al. [53–55]. Using a conjugate of quaternary ammonium with the chemotherapeutic compound melphalan, aggrecan was targeted in an orthotopic Swarm rat chondrosarcoma model. Results showed a reduction of tumor volume for the drug conjugate. The reduction, however, was not significantly different from non-targeted melphalan, although more toxicity was observed for this group indicating an improved toxicity profile for the aggrecan targeting drug conjugate. Despite promising results, care should be taken with possible off-targeting to aggrecan rich tissues such as cartilage, a tissue that was not included in the toxicity evaluations, even though toxicity may be limited due to the limited blood supply to cartilage. Another proteoglycan targeted in the ECM of tumors is heparan sulfate, which is found highly upregulated in ECM of tumors making it an attractive target for tumoral ECM chemotherapeutic drug delivery [56]. Hu et al. used a CGKRK peptide with high affinity to heparan sulfate and conjugated it with an endothelial cell binding peptide to paclitaxel loaded nanoparticles [57]. This strategy was evaluated in mice bearing intracranial glioblastoma tumors and showed that mice treated with paclitaxel loaded nanoparticles targeted against heparan sulfate and endothelial cells significantly improved survival. It is not clear whether the effect is through extracellular release with potential removal of tumor supportive cells or by internalization in tumor cells only. Finally, chondroitin sulfate can be a target in the tumoral ECM because of its high expression in the ECM of various tumor types [58]. Lee et al. used cisplatin loaded liposomes modified with the chondroitin sulfate binding molecule TRX20 (3,5-dipentadecycloxybenzamidine hydrochloride) which showed tumor growth inhibition in a subcutaneous mouse tumor model [59]. While the strategy was designed to target chondroitin sulfate at tumor cell membranes, it may also be applied as ECM targeting to tumors with chondroitin sulfate in the ECM. Our group developed a drug delivery system that targets chondroitin sulfate subtype-E (CS-E), which was found to be highly upregulated in the ECM of ovarian cancer [60]. Although currently only evaluated *in vitro*, doxorubicin loaded albumin particles functionalized with a scFv antibody against CS-E were indeed able to target CS-E and efficiently eliminate ovarian cancer cells by extracellular drug release [20]. Overall, proteoglycans and glycosaminoglycans in the tumoral ECM may offer several opportunities, but care should be taken with off target effects to healthy tissue due to expression of these molecules throughout the body.

### 4.3. Extracellular drug release

Next to the presence of promising targets in the ECM, a tumor ECM drug delivery strategy is also highly dependent on the type of drug carrier. Many chemotherapeutic drug delivery systems have been developed over the last decades. Each system has unique characteristics that can be important for tumoral ECM drug delivery, such as size, drug content, charge, base material, modifications, *etc.* An important characteristic of drug delivery systems for tumoral ECM drug delivery is the drug release mechanism. Upon release, most drug molecules will retain in the tumor area because of the enhanced retention effect and will pass cell membranes due to the hydrophobic properties of the majority of chemotherapeutics. However, if not rapidly taken up by tumor or tumor supportive cells, there is a risk of diffusion back into the circulation, which may result in off-target effects. Unfortunately, preclinical studies generally do not assess reuptake of released drugs into the circulation, but such analyses should be included in future studies. Without sufficient release of chemotherapeutics once a drug delivery system is bound to its target, no therapeutic effect will be induced. Therefore, extracellular release once bound to the ECM of tumors is required. Next to simple diffusion, with possible unwanted preliminary drug release, several innovative release mechanisms have been developed. Examples are triggered release by enzymes, pH, magnetism, heat, light, and ultrasound. For example, by exploiting the lower pH in the tumor extracellular matrix (6.2–6.9) caused by accumulation of lactic acid produced by highly proliferating tumor cells [61], Chiang et al. designed tumor ECM targeting doxorubicin-loaded liposomes in which the imidazole ring of histidine was protonated in an acetic environment resulting in increased uptake of the doxorubicin liposomes [62]. Dong et al. synthesized a pH and enzyme responsive doxorubicin delivery system [63]. The acetic tumor environment exposed the gelatin-DNA-doxorubicin complex to subsequently release doxorubicin by enzymatic degradation of gelatin due to matrix metalloproteinases upregulated in the tumoral ECM. In antibody-drug conjugates, triggered release is applied as well. For example, Rossin et al. developed a non-internalizing antibody-drug conjugate with a click-to-release mechanism [64]. Upon binding to the tumor specific membrane bound target, the non-internalizing antibody-drug conjugate released its payload after reaction of an administered activator compound. This strategy enables release of the drug specifically at the tumor site as non-bound antibody-drug complexes are allowed to be excreted from the body before administration of the activator compound. Next to these examples, a manifold of other release mechanisms have been developed. As thorough discussion of these external/internal stimuli driven response is beyond the scope of this review, we refer to excellent reviews on this topic [61,65,66]. The combination of stimuli triggered release and binding to a tumor ECM target seems a promising idea, but more studies should be performed to indicate its full potential.

## 5. Future outlook

The therapeutic effect of conventional tumor targeting chemotherapeutic delivery systems that addresses molecules on cancer cells may be limited by intratumoral heterogeneity and inadequate drug release due to lysosomal entrapment. Combining the knowledge of tumor heterogeneity and the importance of the tumor extracellular matrix with its tumor supportive cells, delivery of chemotherapeutics to the tumoral ECM may be a promising alternative. Various studies have identified unique tumoral ECM targets. *In vivo* studies indicate that targeting these unique tumoral ECM targets combined with extracellular release of chemotherapeutics can improve treatment outcome. Tumoral ECM targets should be critically selected. Potential expression in healthy tissue may cause off-targeting with possibilities of inducing toxicity and side effects. Moreover, care should be taken when selecting a drug delivery system. The effect caused by extracellular drug release and diffusion of the drug to tumor supportive cells in the tumor area may be limited when the drug as such is not released extracellularly, but instead is contained in a carrier that is taken up into the cell through endocytosis. To overcome these limitations, stimuli driven extracellular drug release may offer promising opportunities. By external or internal triggered drug release, chemotherapeutic agents will only be released in the tumor area and will be able to diffuse into tumor cell and tumor supportive cells. Moreover, it may prevent early drug release that results in exposure to healthy tissue. Next to chemotherapeutic delivery, the emerging field of immunotherapy may greatly benefit from tumor ECM drug delivery. In a study from Zegers et al. [67], the chemokine IL2 was targeted to the tumoral ECM fibronectin extra domain B. Upon radiation, the cytotoxic effect of infiltrating CD8 cytotoxic T lymphocytes was enhanced by the extracellular presence of IL2, illustrating the possibilities to include tumor ECM targeted drug delivery in immunotherapy.

Despite a number of promising *in vivo* results, no clinical studies using tumor ECM targeted chemotherapeutic delivery were identified. Therefore, to understand the full potential of this strategy, the step to clinical studies should be taken once the most potential tumoral ECM

targeting strategy has been identified.

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