Published in final edited form as:

Mol Syst Des Eng. 2017 October 1; 2(4): 370–379. doi:10.1039/C7ME00050B.

Targeted drug delivery using iRGD peptide for solid cancer treatment

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Abstract

Many solid tumor types, such as pancreatic cancer, have a generally poor prognosis, in part because the delivery of therapeutic regimen is prohibited by pathological abnormalities that block access to tumor vasculature, leading to poor bioavailability. Recent development of tumor penetrating iRGD peptide that is covalently conjugated on nanocarriers' surface or co-administered with nanocarriers becomes a popular approach for tumor targeting. More importantly, scientists have unlocked an important tumor transcytosis mechanism by which drug carrying nanoparticles directly access solid tumors (without the need of leaky vasculature), thereby allowing systemically injected nanocarriers more abundantly distribute at tumor site with improved efficacy. In this focused review, we summarized the design and implementation strategy for iRGD-mediated tumor targeting. This includes the working principle of such peptide and discussion on patient-specific iRGD effect in vivo, commensurate with the level of key biomarker (i.e. neuropilin-1) expression on tumor vasculature. This highlights the necessity to contemplate the use of a personalized approach when iRGD technology is used in clinic.

1. Introduction

The development of various nano-enabled drug delivery systems hold the great promise of a fundamental paradigm shift in cancer treatment, both in basic pharmaceutical research and in clinical application.^{1–4} It has been shown that under certain pathological circumstances, including certain solid tumors, the endothelial lining integrity of the tumor vasculature becomes more leaky than normal tissues.^{5–7} This interesting phenomenon serves as the rationale and working principle for the passive tumor targeting, *a.k.a.* enhanced permeability and retention (EPR) effect, by which nanoparticles tend to preferably distribute at tumor sites post systemic administration.^{5–7} While there are concerns that the tumor types and heterogeneity among patients could lead to non-negligible variation in the magnitude of EPR effect, the implementation of this technology has led to FDA-approved nano formulations that positively impact cancer management in patients.^{8–10} However, the EPR effect dependent nanoparticle egress may not be the dominant mechanism in certain tumor types in which the large tumor fenestration is blocked, which can be exemplified by the high pericyte

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coverage on pancreatic tumor vasculature. Another popular particle design approach is active tumor targeting, which frequently refers to the use of ligand–receptor mediated binding affinity to enhance particle uptake.^{11,12} While the early stage of the implementation of active targeting often contains overblown claims of targeted nanoparticles acting as “magic bullets”, the reality is that the enhanced binding affinity relies on close proximity (<0.5 nm), which means that it can only occur after nanoparticle extravasation through the tumor vasculature.¹³ Moreover, it is also important to consider the complexity of active targeting in terms of scale up particle synthesis, quality control, potential for clinical use, abundant and distribution of targeted receptor in patient individual, and the cost of the nanomedicine product. While the calculations remain highly controversial, a recent review paper states that only ~0.7% of the administered nanoparticle dose is found to be delivered to a solid tumor irrespective of active or passive targeting.^{12,13} In order to advance nanomedicine into clinic, it is necessary to further improve nanoparticle tumor targeting efficiency, including the development of alternative and more effective approach for tumor targeting in vivo.

While most nanocarriers that are currently being tested in clinical trials rely on passive and/or active delivery, which depends on the presence of leaky tumor vasculature, there is a complementary approach that involves tumor vascular endothelial cells, which display a network of tubular vesicles (a.k.a. the vesico-vacuolar organelle or VVO) that control vascular access of small molecules, nutrition substances, particulates, and even intact cells.^{16–19} In fact, this complementary mechanism, a.k.a transcytosis, is a unique type of transcellular transport in which various macromolecules (some of which are in nano-size range) are delivered across the interior of cells. For example, endothelial cells in the blood-brain barrier are responsible for brain homeostasis by restricting the access of a variety of compounds, including therapeutic drugs, and yet enable the supply of necessary nutrients, some of which are delivered via endocytosis.²⁰ It was also shown that receptor-mediated transcytosis for peptidic signaling and regulatory molecules (e.g. insulin, leptin, interleukins) and nutrients (e.g. iron, LDL) in different biological processes.²⁰ In the setting of solid tumor, Dr. Erkki Ruoslahti et al., has described an endocytic transcytosis pathway in tumor endothelial cells that can be therapeutically accessed by tumor-penetrating iRGD peptides (CRGD[K/R]GP[D/E]C).^{17,19} While we will discuss the working principle of iRGD below, it suffices to mention here that iRGD is capable of promoting the penetration and tumor cell entry of a range of therapeutics (e.g. free drug, macromolecules, liposomes, and Abraxane[®]) in multiple cancer types.^{15,17,19} In our recent study, we demonstrated this major transcytosis mechanism to complement the classic EPR effect in the almost uniformly fatal pancreatic ductal adenocarcinoma (PDAC).²¹ Unlike particle egression through tumor fenestration, we experimentally demonstrated the use of iRGD peptide to activate transcytosis at the tumor site, leading to an enhanced drug access and efficacy. While there are published review papers to discuss the use of different peptides for cancer diagnosis and therapy,^{21–24} a focused discussion on iRGD-mediated targeting is necessary because it may enhance the tumor targeting via an EPR effect independent fashion, and therefore useful to treat “non-leaky” tumor types. In this focused review, we will summarize the design and implementation strategy for iRGD mediated tumor targeting, including our consideration of personalized nanomedicine when iRGD is used in clinic.

2. iRGD-mediated tumor targeting

2.1 Discovery and working mechanism of iRGD in tumor microenvironment

The tumor-penetrating peptide iRGD was developed by a phage screening method to identify peptides that selectively recognize tumor blood vessels in human prostate cancer animal models.¹⁷ This was achieved through the use of a cyclic CX7C (C, cysteine; X, any amino acid) peptide library displayed on T7 phage for three rounds of ex vivo phage display selection, followed by one round of in vivo selection. The combined ex vivo and in vivo experiments led to new phage pool with 200–400× higher binding affinity to tumor-derived cell suspensions as compared to the average affinity of the original library¹⁷. Three peptide sequences that contained the RGD motif (e.g. CRGDKGPDC, CRGDRGPDC, and CRGDKGPEC) became the most dominant peptides in the new pool. CRGDKGPDC, which binds to PPC1 human prostate cancer cells at 4 °C and can be efficiently internalized by cancer cells at 37 °C, was named as internalizing RGD peptide, *a.k.a.* “iRGD” (see typical structure of the iRGD peptide in Fig. 1). More generally, the cyclic 9-amino peptides with the sequences (CRGD[K/R]GP[D/E]C) are considered iRGD peptides. The working mechanism of the iRGD mediated transport pathway is illustrated in Fig. 2.^{17,19,25–27} The peptide contains two critical sequence motifs, namely an integrin-binding RGD motif and neuropilin-1 (NRP-1) binding motif. The RGD motif mediates the first binding of iRGD to $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrins, which are preferentially overexpressed on tumor blood vessels endothelial cells and tumor cells.^{28,29} Binding of the cyclic peptide to the integrins is followed by a proteolytic cleavage and release of the C-terminal RXXR/K sequence, also dubbed the C-terminal Rule (CendR) motif, which interacts with the NRP-1 receptor. NRP-1 binding leads to the triggering of an endocytic transcytosis and trans-tissue transport pathway that can assist drug and nanoparticle delivery, including small drug molecules, monoclonal antibodies (e.g., trastuzumab), and nanoparticles (e.g., Abraxane®, doxorubicin liposomes and silicasomes).^{19,25,26,30} Since the literature results show that the iRGD technology has been implemented by iRGD covalent coupled approach or iRGD co-administration approach, we have summarized these literature data in the following section.

2.2 Use of covalently conjugated iRGD to improve nanocarriers' tumor targeting

The effectiveness of iRGD-conjugated nanocarriers has been demonstrated in multiple cases that involve a variety of nanoformulations such as liposomes^{31–36}, polymer nanoparticles^{37–43}, polymer nanogels⁴⁴, polymersomes⁴⁵, nanocapsules⁴⁶, exosomes⁴⁷, protein nanoparticles⁴⁸, and inorganic nanoparticles (e.g., iron oxide nanoworms, porous silicon nanoparticles and mesoporous silica nanoparticles)^{49–53}. A detail summary of studies of iRGD conjugated nanocarrier was summarized in Table 1. Depending on the chemical composition, the conjugation reaction frequently involves the use of maleimide-thiol reaction^{17,31–36,38,40–42,44–46,50,52,54}, Michael addition of acryloyl-amine reaction³⁹, alkyne-azide click reaction^{49,51} or amidation of carboxyl-amine reaction^{43,53}, as demonstrated in Fig. 3. This includes the demonstration of tumor targeting effects which were achieved by iRGD conjugation on lipid micelles, Abraxane® (albumin/paclitaxel nanocomplex) and iron oxide nanoworms.¹⁷ In this study, the pristine iRGD peptide was modified with an extra cysteine residue and was conjugated to the nanocarriers through the maleimide-thiol reaction. In the lipid micelle, the iRGD modified lipid was first prepared by coupling the

iRGD to 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-maleimide (polyethylene glycol)_{2,000} (DSPE-PEG_{2,000}), followed by mixing with other lipids that were further used in micelle preparation. For Abraxane[®] and iron oxide nanoworms, the iRGD was conjugated to the particles surface through a sulfo-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate) cross-linker. The maleimide end of the sulfo-SMCC linker selectively reacted with the cysteine sulfhydryl group, whereas the succinimidyl end reacted with the primary amine group on the nano surface. In the subsequent animal studies using orthotopic human prostate, pancreatic and breast cancer models, the authors demonstrated that iRGD conjugation remarkably enhanced the penetration of nanoparticles at the tumor site, multiple hundred μm away from the tumor vasculature as compared to the control particles which primarily distributed in the peri-blood vessel region (Fig. 4). Since then, iRGD surface conjugation has become a popular approach to improve nanocarriers' tumor targeting effect. Take liposomal formulations for example, the effect of iRGD conjugation was intensively studied in the past a few years.^{31–36} The most commonly used conjugation reaction is to use iRGD conjugated DSPE-PEG_{2,000} to make a lipid film, followed by a rehydration process. The iRGD conjugated lipid can be readily made by reacting the cysteine modified iRGD with the commercially available DSPE-PEG_{2,000}-maleimide through the maleimide-thiol reaction.^{17,31} For example, through the iRGD conjugation on 230 nm doxorubicin loaded liposomes, the authors successfully demonstrated facilitated cellular uptake of liposomes and an ~ 2 -fold enhanced antitumor effect in a subcutaneous breast cancer (4T1) model.³¹ Another example was to test the iRGD conjugated doxorubicin liposome in melanoma in which the authors found an $\sim 57.5\%$ improved antitumor efficacy and an $\sim 30\%$ prolonged animal survival (43.5 days vs. 33 days) compared with the non-conjugated liposomes in the highly aggressive B16-F10 skin cancer model.³² In order to achieve the optimal therapeutic outcome, Dai et al. systemically dissected and studied the effect of iRGD density (1 mol% to 10 mol%) on chemo delivery liposomes. This allowed the authors to showed that the liposome with 5 mol% iRGD achieved the best cell uptake and anticancer effect.³³ Beside the classic cyclic iRGD, Liu et al. conjugated the CRGDK peptide, the CendR motif in iRGD, on an irinotecan loaded peptide-crosslinked liposome.³⁴ The data showed that the liposomes exhibited high targeting efficiency in cells with abundant NRP-1 expression, such as HT-29 colon cancer cells, as compared to the cancer type of low NRP-1 expression, such as MCF-7 breast cells. As a result, the CRGDK peptide conjugated liposome led to an improved tumor targeting, penetration and antitumor effect compared to the non-conjugated liposomes in the HT-29 tumor in mice. In addition to the pristine iRGD peptide, iRGD derivatives were reported in the literature.³⁵ Song et al. developed a “tadpole”-like peptide (nRGD) that contained iRGD and a tumor associated macrophage (TAM) targeting sequence (alanine-alanine-asparagine).³⁵ It was demonstrated that conjugating the nRGD to the liposomal carrier could dramatically improve antitumor efficacy, attributed to the specific interaction with tumor vascular endothelial cells capable of efficient tumor penetration, together with the capacity of the depletion of the TAMs in the breast tumor microenvironment. Interestingly, iRGD peptide can also be used to improve the endogenous nano subject for tumor targeting. In this regard, an exosome was used as nanocarrier for doxorubicin encapsulation.⁴⁷ Instead of chemical conjugation, iRGD was integrated into the exosome carrier via a fusion process by plasmid transfection. The iRGD-containing exosomes showed an ~ 3 -fold uptake increase in cultured MDA-MB-231 breast

cancer cells and an 2~5-fold killing effect compared to the non-conjugated exosomes in vitro. Intravenous injection of iRGD-modified exosomes achieved higher tumor distribution and antitumor effect compared to the non-conjugated exosomes in the orthotopic MDA-MB-231 cancer model in mice (Fig. 5).

In the case of polymeric nanoparticles, iRGD was frequently conjugated on the polymer chain during the polymer synthesis through different conjugation reactions as we summarized in Fig. 3.³⁷⁻⁴³ Zhu et al. conjugated iRGD on paclitaxel-loaded PCL-PVP polymer nanoparticles. While iRGD surface modification moderately reduced particle circulation time in the blood stream, the authors demonstrated an improved tumor targeting and antitumor efficacy in a subcutaneous hepatic H22 tumor.³⁷ Another example is demonstrated by Shen et al., who conjugated the iRGD on the paclitaxel/survivin shRNA co-loaded polymer nanoparticle through an acryloyl-amine reaction.³⁹ While there were no significant difference between the nanoparticle cellular uptake with or without iRGD in vitro, use of iRGD conjugation was advantageous in terms of tumor targeting and antitumor effect improvement in an A549/T lung cancer model in nude mice. Similar successes were demonstrated by conjugating iRGD on doxorubicin/sorafenib lipid-polymer hybrid nanoparticle or anti-angiogenesis agent vandetanib laden PEG-PLGA nanoparticle, which were tested in HepG2 liver cancer or BEL-7402 liver cancer, respectively.^{41,42} Moreover, the iRGD conjugated particle was also robustly tested in gene delivery system in different cancer types, such as lung and prostate cancers.^{38,39,43} One example was to use iRGD modification to improve the systemic delivery of siRNA that target survivin. It was shown that the iRGD-conjugated nanoparticle improved the siRNA tumor content for an ~3-fold, which led to an ~3-fold greater knockdown effect in a PC3 prostate cancer xenograft model.⁴³

Recently, the iRGD conjugation was also practiced in the emerging inorganic nanocarriers, such as magnetic core-shell nanoparticles (MCNP), porous silicon nanoparticles, mesoporous silica nanoparticle (MSNP), and iron oxide nanoworms.⁴⁹⁻⁵³ Please note that in certain stroma-rich cancer type, such as a Kras-mutated orthotopic pancreatic cancer model, the iRGD conjugation effect is not less prominent compared to other cancer types.³⁰ Our interpretation is that in addition to the impenetrable pancreatic tumor stroma, the NRP-1 receptor abundance at the tumor vascular site may be the bottle neck that limits the access of conjugated particles to pass through at the tumor site (see section 2.3).

Interestingly, the iRGD conjugated nanocarriers also showed effectiveness when administered intraperitoneally for gastric and colon cancer.⁴⁵ In this regard, the intraperitoneally administered iRGD conjugated paclitaxel-loaded polymersomes showed improved accumulation, penetration and antitumor efficacy compared to the non-conjugated polymersomes.

2.3 Use of co-administrated iRGD to improve nanocarriers' tumor targeting via an activated transcytosis mechanism

While one can practice iRGD technology via conjugation approach, recent advances indicate the possibility of use iRGD plus pharmaceutical products (including nanomedicine) by a co-administration approach.¹⁹ We interpret that as sufficient NRP-1 receptor density to initiate

transmembrane uptake, while the receptor abundance at the tumor vascular site may be more limiting to the number of conjugated particles that dock and are allowed through.^{19,25} Remarkably, the iRGD effect can not only improve the tumor targeting of iRGD conjugated nanocarriers, but can also be effective in a co-administration mode for the nanocarriers that do not couple to the peptide. The peptide can activate a bulk transport system that sweeps along nanocarriers present in the blood.^{19,30,54–61} Sugahara et al. first reported that without chemical conjugation to drugs, intravenous co-administration of iRGD boosted the vascular and tissue permeability in a tumor-specific and NRP-1-dependent manner.¹⁹ The researchers showed that free iRGD increased the tumor access of different drugs, including nano formulations (e.g Nab-paclitaxel and liposomal doxorubicin). With the co-administrated iRGD, these pharmaceutical products more efficiently penetrate into the deep tumor tissue, leading to the significantly enhanced antitumor efficacy in orthotopic human breast tumor (BT474) and/or human prostate tumor (22Rv1) mouse models.¹⁹ Since then, the co-administration approach has been proven in multiple cancer types such as glioblastoma, non-small-cell lung cancer, breast carcinoma, melanoma and pancreatic cancer by different research groups, including our own (Table 2). For example, Agemy et al. found that intravenous co-injecting the iRGD resulted in markedly increased amount of proapoptotic peptide coated iron oxide nanoworms into tumor tissue, with dramatically prolonged mice survival in orthotopic murine glioblastoma mouse model.⁵⁵ Another example was to co-administrate free iRGD with the paclitaxel-loaded PEG-PLA nanoparticles, demonstrating iRGD significantly improved the nanoparticles access across blood-brain tumor barrier and accumulation in glioma parenchyma, which led to longer survival in an orthotopic C6 glioma tumor model.⁵⁷ Furthermore, Sun et al. showed that co-administration with iRGD was able to greatly corroborate the accumulation of paclitaxel loaded red-blood-cell-mimetic nanoparticles in tumor parenchyma, and allowed the nanoparticles to diffuse far away from the blood vessels, with significantly improved tumor growth suppression and decreased lung metastasis compared to PTX-loaded polymer NP alone in a breast 4T1 cancer model.⁶¹

Recently, we showed that the anticancer activity of an irinotecan-loaded silicasome nanocarrier (lipid bilayer coated MSNP^{62,63}) can be significantly improved by the co-administration of an unconjugated iRGD peptide (that does not require the covalent iRGD conjugation to the carrier).³⁰ Through optical imaging and HPLC, we showed that silicasome plus free iRGD increased nanocarrier and payload abundance at orthotopic KPC tumors sites 2~4-fold, which is effective enough to introduce killing enhancement and survival prolonging, as well as metastasis inhibition. In this particular study, we have demonstrated that due to the electron density it is not possible to obtain high resolution ultrastructure images of silica at the tumor site, but have demonstrated that entrapment of a gold core in the silicasome can clearly show the transcytosis and lodging of our drug-laden silicasome in the tumor vasculature, interstitial tissues and cancer cells in the orthotopic pancreatic tumor site (Fig. 6A).³⁰ More importantly, similar improved iRGD-mediated silicasomes tumor accumulation was achieved in patient-derived tumor with high NRP-1 expression on tumor blood vessels, but not in the patient-derived tumor with low NRP-1 expression (Fig. 6B).³⁰

3. Perspective and conclusion

While both the conjugation approach and co-administration approach have been used to improve tumor accumulation, only a few studies provided side-by-side comparisons in the same study. Sugahara et al., showed the tumor accumulation of iRGD-conjugated abraxane is slightly less than that of abraxane co-administrated with iRGD in breast and prostate cancer. The authors didn't obtain the statistical significance between the two groups.¹⁹ Recently, we showed that the co-administration approach is approximately 2.5× more effective than the conjugation approach in Kras-mutated or patient derived pancreatic cancer bearing mice, receiving an IV injected silica-based nanocarrier.³⁰ In our opinion, there are obvious advantages for the co-administration strategy from pharmaceutical activity and nano manufacture perspectives. The co-administration may address a major limitation of peptide-conjugated nanocarriers, which rely on the available number of NRP-1 receptors that transport of the conjugated nanocarrier may be limited by the relatively small and finite number of target receptors on the vasculature, but separate injection of the free peptide can trigger bulk transfer of nanocarriers through the bystander (in greater number) at the tumor site.^{19,25} Moreover, introducing covalently conjugated peptide may add complexity to surface properties of nanocarriers that may lead to undesired impact in the complicated *in vivo* system.^{64,65} It is possible that peptide conjugation, directly or indirectly, leads to particle opsonization and increased uptake by the reticuloendothelial system (RES).^{66,67} The faster uptake by RES organs will result in shorter blood circulation time, ultimately leading to lower tumor accumulation.^{68,69} From the perspective of translational feasibility, the use of the free peptide is more practical and less expensive for clinical use compared to a conjugation process that inevitably enhances the cost and complexity of the nanocarrier.

Recently, there has been high-level coverage on the reproducibility project that aims to duplicate major experimental results in the field of cancer. One of the projects chosen, based on a prominent iRGD effect, was to duplicate the results of iRGD peptide co-administration for enhancing chemotherapy efficacy in a prostate cancer model.⁷⁰ In spite of the fact that multiple international laboratories and our own study have validated the effect, a report showed that the authors executing that study failed to reproduce the iRGD co-administration results.^{70–72} While there can be many explanations for this discrepancy, some important possibilities include the verification of the biological activity of the iRGD peptide, as well as a demonstration of the actual presence of the NRP-1 receptor in the tumor model in order to obtain a valid comparison. In fact, our patient-derived model data suggested that the enhancing effect of the iRGD peptide could vary depending on the level of NRP-1 expression which is different from patient to patient and from one tumor model to another. All things considered, it is necessary to contemplate a personalized nano therapeutic approach, especially in regards to pancreatic cancer (but also for other cancer types), to enhance the efficacy of cancer drug through iRGD co-administration.

Acknowledgments

We would like to acknowledge the financial support from the U.S. Public Health Service Grant 1U01CA198846.

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iRGD: c(CRGDKGPDC)

Fig. 1.
Typical chemical structure of cyclic iRGD peptide.

Fig. 2.
 Schematic of the iRGD activated transcytosis mechanism for nanocarriers delivery in tumor.

Fig. 3.
Summary of the major bioconjugation reactions for the covalent iRGD attachment on nano surface.

Fig. 4.

In vivo tumor penetration of abraxane conjugates with or without iRGD conjugation. Confocal microscopy images of 22Rv1 orthotopic tumors from mice injected with the indicated abraxane conjugates at a paclitaxel equivalent of 3 mg/kg after 3 hrs. iRGD-conjugated abraxane showed more abundant accumulation and deeper penetration far away from the blood vessels compared to control peptide CRGDC conjugated or non-conjugated abraxane. Green, abraxane; red, CD31; blue, DAPI. Scale bars = 100 μ m. Adapted with permission from ref. 17.

Fig. 5.

In vivo tumor targeting ability of iRGD containing exosomes (iRGD-Exos) and antitumor activity of Dox loaded iRGD-Exos (iRGD-Exos-Dox) in MDA-MB-231 tumor bearing mice. (A) In vivo fluorescence imaging of mice after given a single intravenous injection of DiR-labeled blank-Exos or iRGD-Exos. Maximal fluorescence was detected at the tumor sites (white boxes) at 2 h. Two hours after injection of iRGD-Exos, but no fluorescence was associated with the tumors of blank-Exos-treated mice at any time point. (B) Ex vivo fluorescence imaging of major organs from tumor-bearing mice 2 h after intravenous injection with DiR-labeled blank-Exos or iRGD-Exos. (C) Tumor growth rate of mice treated by different reagents (PBS, iRGD-Exos, Free Dox (3 mg/kg), or an equivalent amount of Dox incorporated into blank-Exos (blank-Exos-Dox) or iRGD-Exos (iRGD-Exos-Dox)) every other day for a total of 6 injections (arrows). **, $p < 0.01$; *, $p < 0.05$. Adapted with permission from ref. 47.

Fig. 6.

(A) Ultrastructural TEM visualization of silicasome transcytosis mediated by iRGD co-administration in orthotopic pancreatic tumor. The electron micrograph shows Au labeled silicasomes in (i) the lumen of a tumor blood vessel (red arrows), (ii) transport in the endothelial vesicles (pink arrow), and (iii) deposition in the tumor interstitium (blue arrows). High-magnification images of regions 1 through 3 are provided in the panels on the right. E, endothelial cell; P, pericyte. Scale bar: 2 μ m (left panel); 50 nm (right panels). (B) iRGD-induced silicasome tumor accumulation in patient-derived xenografts. A pair of tumors (XWR#8 and XWR#187) with different levels of NRP-1 expression was selected for a biodistribution study in the absence and presence of iRGD co-administration. Multicolor IHC staining (left panel, green fluorescent antibody for NRP-1, red fluorescent antibody for CD31) demonstrated the relative higher abundance of NRP-1 expression and higher extent of overlap with blood vessels endothelial cells for model XWR#187 than that of model XWR#8. ICP-OES measurement (right panel) showed the uptake of silicasomes in tumors was significantly improved by iRGD co-administration in model XWR#187 but not in model XWR#8. Data represent mean $\pm$ SD. * P < 0.05, 2-tailed Student's t test. Adapted with permission from ref. 30.

iRGD mediated nanocarrier targeting through conjugation. As a general trend, the representative studies summarized in this table showed iRGD conjugated nanoparticles can be more effectively delivered at tumor site. This is usually accompanied with the improved antitumor efficacy in different cancer types.

Table 1

NP type	Conjugation chemistry	Size	Zeta potential	Cancer type	Ref
Lipid micelles Abraxanes (albumin embedded Paclitaxel) Iron oxide nanoworms	Maleimide-thiol reaction	15–25 nm 130 nm 80 × 30 nm	n/a	Orthotopic human prostate PC-3, PPC1 and 22Rv1), pancreatic (MIA PaCa-2) and breast (BT474) cancers	17
Paclitaxel-loaded PCL-PVP polymer nanoparticles	Thiazolidine ring	40–50 nm	–5–10 mV	Subcutaneous murine hepatic H22 tumor	37
siRNA-loaded PLGA-PLL-PEG polymer nanoparticles	Maleimide-thiol reaction	~150 nm	n/a	Subcutaneous human non-small-cell lung cancer (A549)	38
Doxorubicin loaded crosslinked multilayer liposome	Maleimide-thiol reaction	~230 nm	n/a	Subcutaneous murine breast cancer (4T1)	31
Doxorubicin loaded gold nanoclusters conjugated polymer nanogel	Maleimide-thiol reaction	~182.4 nm	–19.43 mV	Murine melanoma B16 cells	44
Doxorubicin loaded liposome	Maleimide-thiol reaction	~90 nm	–14.86 mV	Subcutaneous murine melanoma B16-F10 tumor	32
Paclitaxel and survivin shRNA co-loaded polymer nanoparticle	Acryloyl-amine reaction	141–160 nm	+30 mV	Subcutaneous human non-small-cell lung cancer (A549/T)	39
Doxorubicin loaded exosome	Fused on exosome by plasmid transfection	~97 nm	n/a	Orthotopic human breast MDA-MB-231 tumor	47
Sorafenib loaded porous silicon nanoparticles	Azide-alkyne cycloaddition	~188.8 nm	+11.2 mV	Human endothelial EA.hy926 cells	49
Doxorubicin loaded liposome	Maleimide-thiol reaction	~95 nm	–1.59 mV	subcutaneous murine melanoma B16 tumor	33
Magnetic core-shell nanoparticles (MCNP)	Maleimide-thiol reaction	46.8 nm	+15.78 mV	Human glioblastoma (U87vIII) and breast cancer (MDA-MB-231, MCF-7) cells subcutaneous esophageal KYSE tumor	50
Doxorubicin loaded chitosan-co-PLA/DPPE polymer nanoparticles	Maleimide-thiol reaction	229.9 nm	–12.8 mV	Human endothelial HUVEC and breast carcinoma MB-MDA-231 cells, murine breast carcinoma 4T1 cell	40
Doxorubicin conjugated PAMAM dendrimer	Maleimide-thiol reaction	~22 nm	+2.45 mV	Orthotopic rat C6 glioma tumor in mouse	54
Irinotecan loaded peptide-crosslinked liposome	Maleimide-thiol reaction	~72 nm	+5.7 mV	Subcutaneous human colon HT-29 tumors	34
OSU03012 loaded protein nanocages	Fusion of iRGD to protein using E. coli protein expression system	12–14 nm	n/a	Human pancreatic (AsPC-1, MIA-PaCa2 and Suit-2), colon (HT-29), and breast (MCF-7) cancer cells	48
Sorafenib loaded porous silicon nanoparticles	Azide-alkyne cycloaddition click chemistry	202.8 nm	–23.7 mV	Subcutaneous human prostate PC3-MM2 cancer tumor	51
Iron oxide nanoworms	Maleimide-thiol reaction	~70 × 30 nm	n/a	Metastasis human breast 231BR tumor, murine breast 4T1-BR5 tumor	52

NP type	Conjugation chemistry	Size	Zeta potential	Cancer type	Ref
Doxorubicin and sorafenib co-loaded lipid-polymer hybrid nanoparticles	Maleimide-thiol reaction	~126 nm	-21.4 nm	Subcutaneous human hepatocellular HepG2 tumor	41
Paclitaxel loaded core-shell nanocapsules	Maleimide-thiol reaction	~196.3 nm	-28.63 nm	Subcutaneous murine hepatoma H22 tumor	46
Doxorubicin loaded liposomes	Maleimide-thiol reaction	~150-170 nm	-11.4~ -13.6 mV	Orthotopic murine breast 4T1 tumor	35
Paclitaxel loaded polymersomes	Maleimide-thiol reaction	~233 nm	-2.7 mV	Peritoneal or subcutaneous human gastric (MKN-45P) and murine colon (CT26) tumors	45
Vandetanib loaded PEG-PLGA nanoparticle	Maleimide-thiol reaction	39.8 nm	n/a	Subcutaneous human hepatocellular BEL-7402 tumor	42
Indocyanine green (ICG) loaded liposomes	Maleimide-thiol reaction	115.9 nm	-34.21 mV	Subcutaneous murine breast 4T1 tumor	36
Antiangiogenic (combretastatin A4) and chemotherapeutic (doxorubicin) co-loaded mesoporous silica nanoparticle (MSN)	Amide reaction	~70 nm	~-7 mV	Subcutaneous human cervical HeLa tumor	53
Survivin siRNA loaded polymer nanoparticles	Amide reaction	60~90 nm	+3 ~ +9 mV	Subcutaneous human prostate PC3 tumor	43
Lipid coated mesoporous silica nanoparticle (silicasome)	Maleimide-thiol reaction	~130 nm	~-10 mV	Orthotopic murine pancreatic KPC-derived tumor	30

iRGD mediated nanocarriers targeting through co-administration mode. As a general trend, co-administration of nanocarrier plus non-conjugated iRGD improves drug delivery and antitumor efficacy in different cancer types, such as breast, prostate and pancreatic cancer. The effect of free iRGD is achieved by an activated transcytosis process, which mechanistically differs from the classic EPR effect, which requires enlarged fenestration in the tumor vasculature.

Table 2

NP type	Dose/Treatment procedure	Size	Zeta potential	Cancer type	Ref
Nab-paclitaxel nanoparticle	4 $\mu\text{mol/kg}$	~130 nm	n/a	Orthotopic human breast tumor (BT474)	19
Doxorubicin liposomes	After NP injection	~120 nm	n/a	Orthotopic human prostate tumor (22Rv1)	
Proapoptotic peptide coated iron oxide nanoworms	4 nmol/kg Mixed with NP	80–100 $\times$ 30 nm	n/a	Orthotopic murine glioblastoma 005 tumor	55
Cisplatin loaded mPEG-b-PLG copolymer nanoparticles	4 mg/kg Mixed with NP	10–52 nm	~ 7.82 mV	Subcutaneous human non-small-cell lung cancer (A549)	56
Paclitaxel-Loaded PEG-PLA polymer nanoparticles	4 $\mu\text{mol/kg}$ After NP injection	~131.3 nm	~ -31.43 mV	Orthotopic human C6 glioma tumor	57
Iron Oxide nanoparticles	n/a n/a	n/a	n/a	Human pancreatic cancer Panc-1 cells	58
Doxorubicin conjugated PAMAM dendrimer	4 $\mu\text{mol/kg}$ Mixed with NP	~22 nm	~ +2.45 mV	Orthotopic rat C6 glioma tumor in mouse	54
Doxorubicin-conjugated AuNPs modified on gelatin nanoparticles	4 $\mu\text{mol/kg}$ Mixed with NP	~131 nm	~ -10.3 mV	Subcutaneous murine breast carcinoma 4T1 cell	59
Doxorubicin loaded liposome	4 $\mu\text{mol/kg}$ Mixed with NP	~90 nm	~ -14.86 mV	Subcutaneous murine melanoma B16-F10 tumor	60
Paclitaxel loaded red-blood-cell-mimetic nanoparticles composed of a polymeric core and a RBC vesicle shell	4 $\mu\text{mol/kg}$ Mixed with NP	~147.9 nm	~ -16.1 mV	Orthotopic murine breast 4T1 tumor	61
Irinotecan loaded lipid coated mesoporous silica nanoparticle (silicasome)	8 $\mu\text{mol/kg}$ Mixed with NP	~130 nm	~ -10 mV	Orthotopic murine pancreatic KPC-derived tumor Subcutaneous pancreatic cancer patient-derived tumors	30