Published in final edited form as:

Small. 2014 May 28; 10(10): 1887–1893. doi:10.1002/sml.201303627.

Tumor Vasculature Targeting: A Generally Applicable Approach for Functionalized Nanomaterials

Dr. Feng Chen and

Department of Radiology, University of Wisconsin – Madison, WI, USA

Prof. Weibo Cai

Department of Radiology, University of Wisconsin – Madison, WI, USA. Department of Medical Physics, University of Wisconsin – Madison, WI, USA. University of Wisconsin Carbone Cancer Center Madison, WI, USA, Fax: (+1) 608-265-0614

Feng Chen: FChen@uwhealth.org; Weibo Cai: WCai@uwhealth.org

Abstract

The last decade has witnessed an unprecedented expansion in the design, synthesis and preclinical applications of various multifunctional nanomaterials. Efficient targeting of these nanomaterials to the tumor site is critical for delivering sufficient amount of anti-cancer drugs to suppress tumor growth, while avoiding undesired side effects. Although some nanoparticles could accumulate in the tumor tissue based on the enhanced permeability and retention effect, which may also bind to targets on the tumor cell surface after extravasation from the tumor vasculature, these strategies have many limitations. In this article, we discuss the concept of tumor vasculature targeting and summarize representative examples of in vivo targeted positron emission tomography imaging of various functionalized nanomaterials with different morphology, size and surface chemistry. The concept of targeting tumor vasculature instead of (or in addition to) tumor cells will continue to inspire the design of more advanced nanosystems for efficacious and personalized treatment of cancer in the future.

1. Introduction

Nanotechnology, an interdisciplinary research field which involves chemistry, engineering, biology, medicine, etc., holds tremendous potential for future early detection, accurate diagnosis, and personalized treatment of various diseases such as cancer.^[1] The last decade has witnessed an unprecedented expansion in the design, synthesis and preclinical applications of various multifunctional nanomaterials, which could not only be potentially used for locating cancers early and non-invasively, but also deliver sufficient amount of anti-cancer drugs on-demand to suppress tumor growth.^[2,3] To achieve accurate early cancer diagnosis and/or effective therapeutic outcome, these nanomaterials generally need to be efficiently delivered to the target of interest after intravenous (i.v.) administration for tumor targeted imaging and drug delivery, which faces many challenges.

Certain nanoparticles could accumulate in the tumor tissue by taking advantage of the pathophysiologic characteristics of tumor blood vessels. As shown in Figure 1, in comparison with normal blood vessels, which have well-organized arrangement of arterioles, capillaries, and venules, fast growing tumor vessels are structurally and functionally abnormal with uneven diameter, excessive branching and shunts.^[4] Tumor vessels are also known to have high vascular permeability and lack functional lymphatics due to the uncontrolled growth rate and the changes in endothelial cell shape, resulting in the accumulation of various nanoparticles (typically with sizes less than 300 nm) in tumor tissues based on the enhanced permeability and retention (EPR) effect.^[5] However, because different host organs could produce different pro- and anti-angiogenic molecules, which could lead to tremendous heterogeneity in tumor vessel leakiness over space, time, and different types of tumors,^[6] such passive targeting strategy has its limitations and may only work in certain fast growing tumor models for nanoparticles with relatively long blood circulation lifetime.

Aside from the passive targeting strategy based on the EPR effect, tumor cell targeting is an alternative approach, which could be achieved by chemical conjugation of specific ligands (e.g., antibodies, peptides, small molecules, etc.) to nanoparticles to recognize and selectively bind to their receptors that are overexpressed on certain tumor cell surface (e.g., epidermal growth factor receptor [EGFR], human epidermal growth factor receptor-2 [HER-2], transferrin receptor, folate receptor, etc.).^[8] The high surface area-to-volume ratio of nanoparticles could allow high local density of these ligands for better targeting efficiency. However, tumor cell targeting can only work if the functionalized nanoparticles are able to effectively reach the tumor cell surface after extravasation across the vasculature endothelium. In a recent study, it was concluded that the general rules that govern the extravasation of nanoparticles still remain largely unknown, and nano-particles with very similar surface coating and charge could display surprisingly different extravasation behavior in vivo.^[9] For example, both quantum dots (QDs) and single-walled carbon nanotubes (SWNTs) show virtually no extravasation in xenograft SKOV-3 tumors. QDs were found to extravasate more efficiently than SWNTs in LS174T tumors, whereas the opposite was observed in U87MG tumors.^[9] Even if nanoparticles with suitable shape, size and surface modification could efficiently extravasate, they still have to face other biological barriers (e.g., high interstitial fluid pressure, dense collagen matrix, etc.) and must penetrate tens to hundreds of micrometers before reaching the tumor cell surface and binding to the target of interest.^[10] All of these challenges contribute to the fact that most tumor cell targeted nanoparticles showed highly attractive targeting efficiency in vitro, but rarely exhibited significantly higher uptake in solid tumors when compared with the non-targeted counterparts in vivo.^[11]

Tumor vasculature targeting (i.e., targeting receptors overexpressed on the tumor vascular endothelial cells) is another strategy, which could be generally applicable for a wide variety of well-functionalized nanoparticles regardless of tumor types. Firstly, all solid tumors depend on angiogenesis, the formation of new blood vessels, indicating that targeting tumor angiogenesis/vasculature could be generalized to most solid tumors in vivo.^[12] Secondly, unlike tumor cells which are far away from blood vessels with regard to the size of nanoparticles, tumor endothelial cells are directly exposed to circulating blood, which can

greatly facilitate the binding of functionalized nanoparticles without the barrier of extravasation. Thirdly, many proteins that are overexpressed on tumor vascular endothelial cell surface could be exploited for tumor targeting with drug-loaded nanoparticles, thereby opening up new possibilities for targeted molecular imaging and therapy of cancer.

In this article, we discuss the concept of tumor vasculature targeting and summarize representative examples of in vivo targeted imaging with functionalized nanoparticles, including but not limited to, QDs, SWNTs, nanographene oxide (GO), mesoporous silica nanoparticles (MSN), etc. Positron emission tomography (PET) imaging is an attractive technique that can provide researchers with highly sensitive and quantitative evaluation of the in vivo tumor targeting efficacy, which is also clinically relevant for cancer patient management because of superb tissue penetration of signal. Although tumor vasculature targeting of nanoparticles by using other imaging modalities (e.g., optical imaging, magnetic resonance imaging [MRI], etc.) has also been well-documented in the literature,^[13] due to the page limit and scope of this article, we will focus primarily on radiolabeled nanomaterials that have been designed to target three extensively investigated proteins involved in tumor angiogenesis: vascular endothelial growth factor receptors (VEGFRs), integrins $\alpha_v \beta_3$ and CD105 (endoglin). We will also discuss limitations and future research directions of tumor vasculature targeting with nanomaterials for improved image-guided drug delivery and cancer therapy.

2. Tumor Angiogenesis

It is generally recognized that without newly formed blood vessels for supplying oxygen and nutrients, small solid tumors cannot grow beyond 1–2 mm.^[14] Tumor angiogenesis is characterized by the invasion, migration and proliferation of smooth muscle and endothelial cells, which is dependent on the balance between pro-angiogenic molecules (e.g., vascular endothelial growth factor [VEGF]) and anti-angiogenic molecules (e.g., angiostatin and endostatin).^[6] Tumor angiogenesis occurs as a series of events. First, diseased tissue produces and releases angiogenic growth factors that diffuse into the nearby tissue.^[15] When the angiogenic growth factors bind to specific receptors located on the endothelial cells of pre-existing blood vessels, the endothelial cells become activated and various signaling cascades can lead to the production of new molecules by the endothelial cells.^[16] These molecules can cause tiny holes in the basement membrane surrounding the blood vessels and the endothelial cells begin to proliferate and migrate towards the tumor tissue.^[16] Next, additional enzymes such as matrix metalloproteinases (MMPs) are produced to dissolve the tissue in front of the sprouting vessel tip.^[17] The sprouting endothelial cells can roll up to form individual blood vessel tubes which get connected to form blood vessel loops.^[6,18] Lastly, the newly formed blood vessel tubes are stabilized by specialized muscle cells which provide structural support and the blood flow begins.^[15] Cancer biomarkers that are selectively overexpressed on tumor vascular endothelial cell surface during such angiogenesis process (e.g., various receptors) are highly desirable for tumor targeting, since they are generally applicable to most, if not all, solid tumors.^[19]

3. Tumor Vasculature Targeting with Functionalized Nanomaterials

Nanoparticles, especially those with attractive optical, magnetic, photothermal properties and drug loading capabilities, hold enormous potential in future cancer nanomedicine.^[1] Molecular imaging, with the definition of “visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems”,^[20] has greatly facilitated and enhanced the way that researchers and clinicians visualize and investigate various complex biological events. Over the last several years, researchers from all around the globe,^[21,22] including our own laboratory, have been actively investigating the functionalization of various types of nanomaterials (Figure 2) for tumor vasculature targeting, which could be non-invasively monitored and quantified with PET imaging and/or other techniques.

3.1. Targeting VEGFRs

The VEGF/VEGFR signaling pathway plays a pivotal role in both normal vasculature development and many disease processes.^[19] The angiogenic actions of VEGF are mainly mediated by two endothelium-specific receptor tyrosine kinases: VEGFR-1 and VEGFR-2. VEGFR-1 is critical for physiologic and developmental angiogenesis, and its function varies with the stages of development, the states of physiologic and pathologic conditions, and the cell types in which it is expressed. VEGFR-2 is the major mediator of the mitogenic, angiogenic, and permeability-enhancing effects of VEGF.^[28]

Although in vivo targeted imaging of VEGFR expression using radiolabeled proteins has been well-documented,^[13] few examples of PET imaging with VEGFR-targeted nanoparticles exist in the literature.^[29] In one report, QDs were conjugated with VEGF₁₂₁ and the DOTA chelator (i.e., 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), which were then labeled with ⁶⁴Cu (a PET isotope with a decay half-life of 12.7 h) for VEGFR-targeted PET/near-infrared fluorescence (NIRF) imaging. The DOTA-QD-VEGF₁₂₁ exhibited high VEGFR-2-specific binding affinity in vitro. U87MG tumor accumulation of ⁶⁴Cu-labeled DOTA-QD-VEGF₁₂₁ in vivo was $\approx 4\%$ ID/g at 24 h post-injection (p.i.), significantly higher than that of ⁶⁴Cu-DOTA-QD ($< 1\%$ ID/g) which only takes advantage of the EPR effect. Good correlation was observed between the results measured by ex vivo PET and NIRF imaging, indicating that PET imaging closely reflected the biodistribution of these ⁶⁴Cu-labeled QDs. It is worth pointing out that VEGFR expression level could be dramatically different for the same tumor model at different sizes and stages. Small (≈ 60 mm³) U87MG tumors typically have high level of VEGFR-2 expression, while larger ones (≈ 1000 mm³) have significantly lower VEGFR-2 expression level.^[30] Therefore, successful clinical translation of tracers (or functionalized nanoparticles) in the future for non-invasive imaging of tumor angiogenesis, PET image-guided delivery of anti-cancer drugs, monitoring the therapeutic efficacy, etc. is critical for addressing the highly dynamic nature and heterogeneity of solid tumors.

3.2. Targeting Integrin $\alpha_v\beta_3$

Integrins are an important family of cell adhesion molecules that are involved in a wide range of cell-extracellular matrix and cell-cell interactions.^[31] Integrins expressed on

endothelial cells can modulate cell migration and survival during the angiogenesis process, whereas those expressed on cancer cells may potentiate metastasis by facilitating invasion and movement across the vasculature. Among all integrin family members discovered to date, integrin $\alpha_v\beta_3$ is the most intensively studied. With the use of *in vivo* phage display, scientists have identified tumor-homing peptides with an arginine-glycine-aspartic acid (RGD) motif that could bind specifically to integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$,^[32–35] opening up new possibilities for tumor vasculature targeted imaging and drug delivery. Of note, besides being overexpressed on tumor neovasculature, integrin $\alpha_v\beta_3$ is also expressed on a variety of tumor cells (e.g., brain tumor, breast and prostate cancer, melanoma, etc.).^[13,31] Therefore, it is not as specific for tumor vasculature as VEGFR-2 or CD105.

Following the pioneering work on RGD-conjugated QDs for imaging blood and lymphatic vessels,^[36] we reported the *in vivo* NIRF imaging of integrin $\alpha_v\beta_3$ on the U87MG tumor vasculature using similar nanoconjugates.^[24,37] RGD peptides were conjugated to QDs (Figure 2A), which exhibited integrin $\alpha_v\beta_3$ specific binding in cell culture and *ex vivo*. *In vivo* experiments showed clear tumor contrast as early as 20 minutes after *i.v.* injection of QD-RGD, which reached a maximum within a few hours *p.i.* In a follow-up study, a PET/NIRF dual-modality probe was developed which could allow for a better understanding of the *in vivo* pharmacokinetics of QDs and more accurate quantification of tumor targeting efficacy.^[38] The U87MG tumor accumulation of ^{64}Cu -DOTA-QD (i.e., non-targeted) was found to be less than 1%ID/g, which was significantly lower than that of ^{64}Cu -DOTA-QD-RGD (i.e., targeted, $\approx 4\%$ ID/g, Figure 3A). Histologic examination further revealed that ^{64}Cu -DOTA-QD-RGD targeted primarily the tumor vasculature with little extravasation.

Besides active tumor vasculature targeting of functionalized QDs, this strategy has been demonstrated to be applicable to a variety of other nanomaterials of different dimensions and surface coatings. For example, ^{64}Cu -labeled, RGD-conjugated and polyethylene glycol (PEG) coated SWNTs were prepared to investigate the *in vivo* biodistribution pattern and tumor targeting efficiency (Figure 2B, 3B).^[23] PET imaging showed very high uptake (10–15%ID/g) of ^{64}Cu -labeled SWNT-PEG₅₄₀₀-RGD in the integrin $\alpha_v\beta_3$ -positive U87MG tumor, while the non-targeted group (i.e., ^{64}Cu -labeled SWNT-PEG₅₄₀₀) and SWNTs coated with a shorter PEG chain (i.e., SWNT-PEG₂₀₀₀-RGD) showed significantly lower tumor accumulation ($<5\%$ ID/g) at 6 h *p.i.* (Figure 3B). This work highlighted the importance of tumor vasculature targeting for successful delivery of functionalized SWNTs to the tumor sites *in vivo*. Of note, appreciable level of passive targeting was observed for these functionalized SWNTs, tumor accumulation of ^{64}Cu -SWNT-PEG₅₄₀₀-RGD could be attributed to a combination of the EPR effect, tumor vasculature integrin $\alpha_v\beta_3$ targeting (which played a dominant role in this case), as well as tumor cell targeting (since the U87MG tumor cells also express high level of integrin $\alpha_v\beta_3$).

Subsequently, a variety of other RGD-functionalized nanomaterials such as zinc oxide nanowires,^[40] superparamagnetic iron oxide nanoparticles (SPION, Figure 2C, 3C),^[25,41] unimolecular micelles (Figure 2E, 3E),^[26] gold nanorods,^[42] apoferritin,^[43] biodegradable dendrimer,^[44] etc. with varied chemical compositions, particle sizes, and morphologies have also been reported for *in vitro* and *in vivo* targeting of integrin $\alpha_v\beta_3$, which clearly

demonstrated the versatility and broad applicability of such tumor vasculature targeting strategy.

3.3. Targeting CD105

CD105, also known as endoglin, is an ideal marker for tumor angiogenesis since it is almost exclusively expressed on proliferating endothelial cells.^[45] Various literature reports have shown that the expression level of CD105 is correlated with poor prognosis in more than 10 solid tumor types, which makes it an extremely attractive and universal vascular target for solid tumors.^[46] Using TRC105 (a human/murine chimeric IgG1 monoclonal antibody that binds to both human and murine CD105^[47] as the targeting moiety, we reported the first PET imaging of CD105 expression in a mouse model of breast cancer,^[48,49] and subsequently confirmed high CD105-mediated uptake of radiolabeled TRC105 in a number of xenograft tumor models including triple-negative breast cancer, pancreatic cancer, prostate cancer, and brain tumor, demonstrating broad potential for future applications of CD105-targeted nanomaterials in cancer imaging and therapy.^[50]

TRC105-conjugated GO was the first type of nanomaterial that was successfully delivered to the tumor site based on tumor vasculature CD105 targeting.^[39] GO and reduced GO have attracted increasing interest for cancer imaging and therapy over the last several years because of their many intriguing properties (e.g., drug/gene loading capacity, versatile chemistry, photothermal property, etc.).^[51] However, in vivo active tumor targeting has not been achieved for these nanomaterials until recently by our group.^[39,52,53] Using TRC105 as the targeting ligand, we demonstrated that functionalized GO can be specifically directed to the tumor vasculature in vivo via CD105 targeting in the 4T1 tumor model, even when the 4T1 tumor cells are CD105-negative (i.e., exclusively tumor vasculature targeting without tumor cell targeting).^[39] TRC105-conjugated GO (Figure 2D) showed excellent stability and targeting efficacy in vitro. In vivo studies further revealed the significantly higher tumor accumulation for ⁶⁴Cu-NOTA-GO-TRC105 ($\approx 5\%$ ID/g) than that of ⁶⁴Cu-NOTA-GO ($\approx 2\%$ ID/g) at 3 h p.i. (Figure 3D). In addition, CD105 specificity for tumor accumulation of ⁶⁴Cu-NOTA-GO-TRC105 in vivo was confirmed by blocking study and ex vivo histological analysis. Overall, the significantly improved tumor targeting efficiency of TRC105-conjugated GO and reduced GO achieved in these studies^[39,52,53] not only shows great potential for targeted cancer therapy with these GO nanoconjugates, but also offers valuable insights for the future design of various other nanoconjugates for cancer-targeted imaging and therapy.

Our recent research on image-guided drug delivery further demonstrated that CD105-based tumor vasculature targeting could also provide a better approach for designing advanced drug delivery systems.^[27,54] For example, the surface of 80-nm-sized MSN was modified with PEG, (S)-2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (i.e., NOTA), and TRC105 (Figure 2F). After labeling with ⁶⁴Cu, systematic in vitro and in vivo studies clearly indicated CD105-specific targeting of the as-designed ⁶⁴Cu-NOTA-MSN-TRC105 nanoconjugate, with the peak tumor accumulation of $\approx 6\%$ ID/g at 5 h p.i. (Figure 3F).^[27] As a proof-of-concept, we further demonstrated a 2-fold enhancement in targeted delivery of doxorubicin (DOX) to 4T1 tumor-bearing mice after i.v. injection of DOX-

loaded NOTA-MSN-TRC105 nanoconjugate.^[27] Although only hydrophilic drug was tested in this study, TRC105-functionalized MSN could also potentially be used for targeted delivery of hydrophobic drugs, small interfering RNA, etc. to improve their bioavailability and accumulation in the tumor tissue.

3.4. Challenges and Future Directions

Although tumor vasculature targeting has been demonstrated to be a versatile strategy for many well-functionalized nanomaterials regardless of tumor type, many challenges still exist which remain to be conquered in the near future. Firstly, the absolute level of tumor accumulation for most of the nanomaterials investigated to date based on tumor vasculature targeting is still relatively low (typically <10% ID/g), when compared with the liver uptake (usually $\approx$ 20% ID/g or higher for most of the nanomaterials upon i.v. injection). Although it has been generally accepted that factors such as chemical composition of nanoparticles, their sizes, morphologies, surface charge, density of PEG chains and/or targeting ligands, tumor models and tumor locations could affect the biodistribution and accumulation of certain functionalized nanoparticles, general rules regarding how to design the best nanosystem for optimized tumor vasculature targeting remain to be elucidated. To the best of our knowledge, systematic side-by-side comparison of the in vivo tumor vasculature targeting efficiency of specific nanoparticles with varied particle size, morphology, targeting ligand density, etc. does not exist in the literature, which deserves significant research effort in the future. Further optimization of these factors may lead to higher tumor targeting efficiency and significantly improved tumor-to-liver ratio.

Secondly, the existing literature reports on advanced drug delivery systems that target the tumor vasculature have shown exciting potential for targeted cancer therapy, which is now under active development. Further efforts in optimizing the drug loading capacity, improving in vivo targeting efficiency, and accurate monitoring of targeted chemo- and/or photothermal- therapeutic efficacy are also needed. Thirdly, besides the three widely studied angiogenesis-related proteins discussed above, engineering of nanomaterials that can target other proteins on the tumor vasculature^[55], such as vascular cell adhesion molecule-1,^[56] E-selectin,^[57] tumor endothelial marker 8 (TEM8),^[58] etc., have been severely understudied to date. We believe that this research area will witness rapid growth in the near future. Fourthly, since angiogenesis is not only involved in cancer, but also plays an important role in various cardiovascular and inflammatory diseases,^[6] vasculature targeted imaging and drug delivery of well-functionalized nanomaterials to non-cancerous diseases have also been an area of significant interest which warrants more future research effort. Lastly, although hundreds of clinical trials of anti-angiogenic agents for targeting different angiogenesis-related biomarkers are underway,^[59,60] clinical translation of novel multifunctional nanomaterials will continue to be a major hurdle in the future due to many issues (e.g., potential toxicity, high cost, etc.).

4. Conclusion

In many cases, tumor cell targeting alone using functionalized nanomaterials does not exhibit enhanced tumor accumulation when compared with the non-targeted nanomaterials (which is solely based on the EPR effect), since nanomaterials cannot reach the tumor cells

until after extravasation (which is closely related to and primarily responsible for the EPR effect). Based on our previous experience from the studies discussed above and various unpublished data, we conclude that tumor cell targeting alone is unlikely to improve the tumor accumulation of targeted nanomaterials, although it may prolong the tumor retention of these nanomaterials because of specific binding to tumor cells, provided that the tumor accumulation level based on the EPR effect alone is prominent.

In this concise concept article, we discussed tumor vasculature targeting of functionalized nanomaterials with varied particle morphology, size and surface chemistry in different tumor models. Directly targeting tumor endothelial cells (in addition to targeting tumor cells) will alleviate or circumvent the requirement of nanoparticle extravasation, which can be generally applicable to many nanomaterials regardless of tumor types. Representative examples of in vivo tumor vasculature targeted PET imaging of radiolabeled nanomaterials, including QDs, SWNTs, GO, and MSN have been presented, with a main focus on targeting VEGFRs, integrin $\alpha_v\beta_3$, and CD105. We believe that tumor vasculature targeting will continue to inspire researchers in the field of cancer nano-medicine to design more advanced nanosystems in the future, which can give an extra boost to tumor accumulation (when passive targeting based on the EPR effect is prominent) or enable tumor-specific targeting (when EPR effect is negligible) of various novel nanomaterials.

Acknowledgments

This work is supported, in part, by the University of Wisconsin – Madison, the National Institutes of Health (NIBIB/NCI 1R01CA169365), the Department of Defense (W81XWH-11-1-0644), and the American Cancer Society (125246-RSG-13-099-01-CCE).

References

1. Thakor AS, Gambhir SS. CA Cancer J Clin. 2013 ePub.
2. Cai W, Chen X. Small. 2007; 3:1840. [PubMed: 17943716]
3. Ma X, Zhao Y, Liang XJ. Acc Chem Res. 2011; 44:1114. [PubMed: 21732606]
4. McDonald DM, Choyke PL. Nat Med. 2003; 9:713. [PubMed: 12778170]
5. Fang J, Nakamura H, Maeda H. Adv Drug Delivery Rev. 2011; 63:136.
6. Carmeliet P, Jain RK. Nature. 2000; 407:249. [PubMed: 11001068]
7. Dudley AC. Cold Spring Harb Perspect Med. 2012; 2:a006536. [PubMed: 22393533]
8. Byrne JD, Betancourt T, Brannon-Peppas L. Adv Drug Delivery Rev. 2008; 60:1615.
9. Smith BR, Kempen P, Bouley D, Xu A, Liu Z, Melosh N, Dai H, Sinclair R, Gambhir SS. Nano Lett. 2012; 12:3369. [PubMed: 22650417]
10. Nichols JW, Bae YH. Nano Today. 2012; 7:606. [PubMed: 23243460]
11. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW. Cancer Res. 2006; 66:6732. [PubMed: 16818648]
12. Hanahan D, Weinberg RA. Cell. 2011; 144:646. [PubMed: 21376230]
13. Cai W, Chen X. J Nucl Med. 2008; 49(Suppl 2):113S. [PubMed: 18523069]
14. Folkman J. Annu Rev Med. 2006; 57:1. [PubMed: 16409133]
15. Risau W. Nature. 1997; 386:671. [PubMed: 9109485]
16. Carmeliet P. Nat Med. 2000; 6:389. [PubMed: 10742145]
17. Egeblad M, Werb Z. Nat Rev Cancer. 2002; 2:161. [PubMed: 11990853]
18. Bergers G, Benjamin LE. Nat Rev Cancer. 2003; 3:401. [PubMed: 12778130]
19. Ferrara N. Nat Rev Cancer. 2002; 2:795. [PubMed: 12360282]

20. Mankoff DA. J Nucl Med. 2007; 48:18N.
21. Ding Y, Li S, Nie G. Nanomedicine. 2013; 8:1209. [PubMed: 23837858]
22. Yu MK, Park J, Jon S. Theranostics. 2012; 2:3. [PubMed: 22272217]
23. Liu Z, Cai W, He L, Nakayama N, Chen K, Sun X, Chen X, Dai H. Nat Nanotechnol. 2007; 2:47. [PubMed: 18654207]
24. Cai W, Shin DW, Chen K, Gheysens O, Cao Q, Wang SX, Gambhir SS, Chen X. Nano Lett. 2006; 6:669. [PubMed: 16608262]
25. Yang X, Hong H, Grailer JJ, Rowland IJ, Javadi A, Hurley SA, Xiao Y, Yang Y, Zhang Y, Nickles RJ, Cai W, Steeber DA, Gong S. Biomaterials. 2011; 32:4151. [PubMed: 21367450]
26. Xiao Y, Hong H, Javadi A, Engle JW, Xu W, Yang Y, Zhang Y, Barnhart TE, Cai W, Gong S. Biomaterials. 2012; 33:3071. [PubMed: 22281424]
27. Chen F, Hong H, Zhang Y, Valdovinos HF, Shi S, Kwon GS, Theuer CP, Barnhart TE, Cai W. ACS Nano. 2013; 7:9027. [PubMed: 24083623]
28. Ferrara N. Endocr Rev. 2004; 25:581. [PubMed: 15294883]
29. Chen K, Li ZB, Wang H, Cai W, Chen X. Eur J Nucl Med Mol Imaging. 2008; 35:2235. [PubMed: 18566815]
30. Cai W, Chen K, Mohamedali KA, Cao Q, Gambhir SS, Rosenblum MG, Chen X. J Nucl Med. 2006; 47:2048. [PubMed: 17138749]
31. Desgrosellier JS, Cheresch DA. Nat Rev Cancer. 2010; 10:9. [PubMed: 20029421]
32. Pierschbacher MD, Ruoslahti E. Nature. 1984; 309:30. [PubMed: 6325925]
33. Ruoslahti E. Annu Rev Cell Dev Biol. 1996; 12:697. [PubMed: 8970741]
34. Arap W, Pasqualini R, Ruoslahti E. Science. 1998; 279:377. [PubMed: 9430587]
35. Ruoslahti E. Biochem Soc Trans. 2004; 32:397. [PubMed: 15157146]
36. Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E. Proc Natl Acad Sci USA. 2002; 99:12617. [PubMed: 12235356]
37. Cai W, Chen X. Nat Protoc. 2008; 3:89. [PubMed: 18193025]
38. Cai W, Chen K, Li ZB, Gambhir SS, Chen X. J Nucl Med. 2007; 48:1862. [PubMed: 17942800]
39. Hong H, Yang K, Zhang Y, Engle JW, Feng L, Yang Y, Nayak TR, Goel S, Bean J, Theuer CP, Barnhart TE, Liu Z, Cai W. ACS Nano. 2012; 6:2361. [PubMed: 22339280]
40. Hong H, Shi J, Yang Y, Zhang Y, Engle JW, Nickles RJ, Wang X, Cai W. Nano Lett. 2011; 11:3744. [PubMed: 21823599]
41. Lee HY, Li Z, Chen K, Hsu AR, Xu C, Xie J, Sun S, Chen X. J Nucl Med. 2008; 49:1371. [PubMed: 18632815]
42. Xiao Y, Hong H, Matson VZ, Javadi A, Xu W, Yang Y, Zhang Y, Engle JW, Nickles RJ, Cai W, Steeber DA, Gong S. Theranostics. 2012; 2:757. [PubMed: 22916075]
43. Zhen Z, Tang W, Chen H, Lin X, Todd T, Wang G, Cowger T, Chen X, Xie J. ACS Nano. 2013; 7:4830. [PubMed: 23718215]
44. Almutairi A, Rossin R, Shokeen M, Hagooley A, Ananth A, Capoccia B, Guillaudeu S, Abendschein D, Anderson CJ, Welch MJ, Frechet JM. Proc Natl Acad Sci USA. 2009; 106:685. [PubMed: 19129498]
45. Seon BK, Haba A, Matsuno F, Takahashi N, Tsujie M, She X, Harada N, Uneda S, Tsujie T, Toi H, Tsai H, Haruta Y. Curr Drug Delivery. 2011; 8:135.
46. Dallas NA, Samuel S, Xia L, Fan F, Gray MJ, Lim SJ, Ellis LM. Clin Cancer Res. 2008; 14:1931. [PubMed: 18381930]
47. Rosen LS, Hurwitz HI, Wong MK, Goldman J, Mendelson DS, Figg WD, Spencer S, Adams BJ, Alvarez D, Seon BK, Theuer CP, Leigh BR, Gordon MS. Clin Cancer Res. 2012; 18:4820. [PubMed: 22767667]
48. Hong H, Severin GW, Yang Y, Engle JW, Zhang Y, Barnhart TE, Liu G, Leigh BR, Nickles RJ, Cai W. Eur J Nucl Med Mol Imaging. 2012; 39:138. [PubMed: 21909753]
49. Hong H, Yang Y, Zhang Y, Engle JW, Barnhart TE, Nickles RJ, Leigh BR, Cai W. Eur J Nucl Med Mol Imaging. 2011; 38:1335. [PubMed: 21373764]

50. Zhang Y, Hong H, Engle JW, Yang Y, Theuer CP, Barnhart TE, Cai W. Mol Pharm. 2012; 9:645. [PubMed: 22292418]
51. Yang K, Feng L, Shi X, Liu Z. Chem Soc Rev. 2013; 42:530. [PubMed: 23059655]
52. Shi S, Yang K, Hong H, Valdovinos HF, Nayak TR, Zhang Y, Theuer CP, Barnhart TE, Liu Z, Cai W. Biomaterials. 2013; 34:3002. [PubMed: 23374706]
53. Hong H, Zhang Y, Engle JW, Nayak TR, Theuer CP, Nickles RJ, Barnhart TE, Cai W. Biomaterials. 2012; 33:4147. [PubMed: 22386918]
54. Guo J, Hong H, Chen G, Shi S, Zheng Q, Zhang Y, Theuer CP, Barnhart TE, Cai W, Gong S. Biomaterials. 2013; 34:8323. [PubMed: 23932288]
55. Neri D, Bicknell R. Nat Rev Cancer. 2005; 5:436. [PubMed: 15928674]
56. Behm CZ, Kaufmann BA, Carr C, Lankford M, Sanders JM, Rose CE, Kaul S, Lindner JR. Circulation. 2008; 117:2902. [PubMed: 18506006]
57. Hebbar M, Peyrat JP. Int J Biol Markers. 2000; 15:15. [PubMed: 10763135]
58. Gutwein LG, Al-Quran SZ, Fernando S, Fletcher BS, Copeland EM, Grobmyer SR. Anticancer Res. 2011; 31:3417. [PubMed: 21965755]
59. Gupta R, Tongers J, Losordo DW. Circ Res. 2009; 105:724. [PubMed: 19815827]
60. Rosen L. Oncologist. 2000; 5(Suppl 1):20. [PubMed: 10804087]

Biographies

Feng Chen received his PhD degree in Materials Physics and Chemistry from Shanghai Institute of Ceramics, Chinese Academy of Sciences (P.R. China) in 2012. He is currently a Research Associate under the supervision of Prof. Weibo Cai in the Department of Radiology, University of Wisconsin – Madison. Dr. Chen’s research interests involve the design and synthesis of multifunctional nanosystems for cancer targeted imaging and therapy.

Weibo Cai is an Associate Professor in the Department of Radiology at the University of Wisconsin – Madison. He received a PhD degree in Chemistry from UCSD in 2004. After post-doctoral training at Stanford University, he launched his career at UW – Madison in early 2008 and was recently promoted to Associate Professor with Tenure. His research at UW – Madison is primarily focused on molecular imaging and nanotechnology (<http://>

mi.wisc.edu/), investigating the biomedical applications of various agents developed in his laboratory for imaging and therapy of cancer and cardiovascular diseases.

Figure 1.

Differences between normal and tumor blood vessels. (A) A scanning electron microscopy (SEM) image of polymer cast of normal microvasculature, showing simple, organized arrangement of arterioles, capillaries, and venules. (B) A SEM image of polymer cast of tumor microvasculature, showing disorganization and lack of conventional hierarchy of blood vessels. Reproduced with permission.^[4] Copyright 2003, Nature Publishing Group. Arterioles, capillaries, and venules are not identifiable. Schematic illustrations of normal endothelial cells (C) and tumor endothelial cells (D) are also shown. Normal endothelial cells form tight junctions with one another without overlapping at the margins, while tumor endothelial cells branch and sprout excessively, resulting in a defective endothelial monolayer and loss of normal barrier function. Reproduced with permission.^[7] Copyright 2012, Cold Spring Harbor Laboratory Press.

Figure 2.

Representative nanomaterials with varied size and morphology that have been reported for tumor vasculature targeting. Atomic force microscopy (AFM) images of (A) QD-RGD which is sphere-shaped of ≈ 20 nm in diameter. Reproduced with permission.^[24] Copyright 2006, American Chemical Society. (B) SWNT-PEG which has tube-like morphology of 1–5 nm in diameter and 100–300 nm in length. Reproduced with permission.^[23] Copyright 2007, Nature Publishing Group. (D) NOTA-GO-TRC105 which has sheet-like morphology of 20–80 nm in each dimension. Reproduced with permission.^[39] Copyright 2012, American Chemical Society. Transmission electron microscopy (TEM) images of (C) NOTA-SPION-RGD which is irregularly shaped of ≈ 10 nm in size. Reproduced with permission.^[25] Copyright 2011, Elsevier. (E) Unimolecular micelles (NOTA-H40-DOX-RGD) which are spherically shaped of 22–31 nm in diameter. Reproduced with permission.^[26] Copyright 2012, Elsevier. (F) NOTA-MSN-TRC105 which is spherically shaped of ≈ 80 nm in diameter. Reproduced with permission.^[27] Copyright 2012, American Chemical Society^[25]

Figure 3.

A schematic illustration showing the effect of tumor vasculature targeting to three different proteins, using differently functionalized nanomaterials. (A) PET images of ^{64}Cu -labeled quantum dots in U87MG tumor-bearing mice, with or without the use of RGD peptides to target integrin $\alpha_v\beta_3$. Reproduced with permission.^[38] Copyright 2007, Society of Nuclear Medicine and Molecular Imaging. (B) PET images of ^{64}Cu -labeled single-walled carbon nanotubes in U87MG tumor-bearing mice, with or without the use of RGD peptides to target integrin $\alpha_v\beta_3$. Reproduced with permission.^[23] Copyright 2007, Nature Publishing Group. (C) PET images of ^{64}Cu -labeled superparamagnetic iron oxide nanoparticles in U87MG tumor-bearing mice, with or without the use of RGD peptides to target integrin $\alpha_v\beta_3$. Reproduced with permission.^[25] Copyright 2011, Elsevier. (D) PET images of ^{64}Cu -labeled nanographene oxide in 4T1 tumor-bearing mice, with or without the use of TRC105 to target CD105. Reproduced with permission.^[39] Copyright 2012, American Chemical Society. (E) PET images of ^{64}Cu -labeled unimolecular micelles in U87MG tumor-bearing mice, with or without the use of RGD peptides to target integrin $\alpha_v\beta_3$. Reproduced with permission.^[26] Copyright 2012, Elsevier. (F) PET images of ^{64}Cu -labeled mesoporous silica nanoparticles in 4T1 tumor-bearing mice, with or without the use of TRC105 to target CD105. Tumors were indicated by arrows or arrowheads in all cases. Reproduced with permission.^[27] Copyright 2012, American Chemical Society.