Journal of Controlled Release 153 (2011) 198–205

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Perspective

Targeted drug delivery to tumors: Myths, reality and possibility

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1. Introduction

The ultimate goal of drug delivery research is to help patients by developing clinically useful formulations. During the last several decades controlled drug delivery technology has advanced significantly, leading to the development of various clinical formulations improving patient compliance and convenience [1]. Current technologies allow delivery of drugs at desired release kinetics for extended periods of time ranging from days to years. Oral and transdermal drug delivery systems routinely deliver drugs for 24 h, substantially improving drug efficacy and minimizing side effects. Implantable systems can locally deliver drugs for months, even years. While significant advances have been made, there are still areas where substantial improvements need to be made to reach the next level of clinical relevance. One such area is targeted drug delivery to solid tumors. The clinically significant impact of targeted drug delivery lies in the ability to specifically target a drug or drug carrier to minimize drug-originated systemic toxic effects.

Successful translation (from bench to bedside) of potential cancer and gene therapies, particularly small interfering RNA (siRNA) delivery, will largely depend on targeted drug delivery strategies. Overcoming the many challenges of identifying a successful targeted drug delivery strategy requires an understanding of events involving transport of drug or drug carrier to a target site after intravenous (i.v.) administration as well as issues relevant for specific target diseases and the body's response toward a drug delivery system. The current lack of clear recognition of problems facing the drug delivery field can be anticipated to result in only marginal advances in targeted drug delivery technologies in the coming years. The current unmet needs and challenges in this area were summarized by Professor Alexander T. Florence who is one of the few who raised awareness on the exaggerated claims of the nanoparticle-based drug targeting [2,3]. They need to be better appreciated and understood for achieving greater success in drug targeting to tumors. Thus, it would be profitable to address a variety of issues and factors that could affect the development of improved targeted drug delivery systems. Many terms have been used to describe nano-sized drug delivery systems, and here the term “nanoparticle” is used to represent a spectrum of systems, including nanocarrier, nanovehicle, nanosystem, nanostructure, and other terms used in the literature.

2. A few observations on anticancer treatment

A typical *in vitro* study of targeted anticancer drug delivery is based on cultured human cancer cells which express a unique surface marker specifically selected to test the targeted delivery strategy being examined. Cytotoxicity is commonly examined by the addition of a drug delivery system directly to cells grown as a monolayer or in suspension. Such studies produce a dose–response curve with an IC_{50} (the concentration needed to inhibit 50% cell growth) of an anticancer agent under these *in vitro* conditions. The IC_{50} values determined from the *in vitro* studies, however, are found to be difficult to predict therapeutic efficacy in clinical settings [4].

Initial *in vivo* testing of most targeted drug delivery technologies are performed using human cancer cell xenografts established in severe combined immunodeficiency (SCID) mice [4,5]. Alternately, mice having specific genetic alteration which leads to the onset of an oncogenic event are used [4,5]. Reports in the literature commonly describe drug delivery systems that show a substantial, and thus promising, decrease in tumor size. In most of these studies, however, there is an incomplete eradication of these solid tumors and tumor size rapidly increases once the treatment has been stopped [6]. Consistent with these observations in small animal models, there has been little, if any, translation of promising *in vivo* outcomes to studies in man [7].

Translation of promising pre-clinical approaches to clinical trial success has been poor at best and may relate to striking differences in environmental aspects and disease status at the time of treatment. Cancer patients who receive conventional chemotherapy after debulking surgery, which kills rapidly proliferating cells including residual cancer cells, suffer from hair loss, stomach irritation, and low counts of blood cells. When the chemotherapy is stopped, the patients start recovering hair, apatite, and blood cell counts. It is thought that the somatic adult stem cells of the organs survive the chemotherapy and are able to replace healthy cells lost due to these protocols. Could cancer cell recurrence be similar to the recovery process of normal healthy cells? If so, do we need to target the cancer stem cells and how could this be done effectively? Thus, complete cancer cell eradication may be possible using treatments that effectively target both cancer cells and their progenitor stem cells. As stem cells for various tissue cell types do not always reside in that tissue or organ [8], the strategies to effectively target drugs to both tumor and non-tumor sites may be what is required.

3. Targeted drug delivery

The term “targeted drug delivery” (or “drug targeting”) used in drug delivery is distinct from “targeted therapy” (or “targeting

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doi:10.1016/j.jconrel.2011.06.001

therapy") that is frequently used in drug discovery. Targeted drug delivery refers to predominant drug accumulation within a target zone that is independent of the method and route of drug administration [9]. On the other hand, targeted therapy or targeted medicine means specific interaction between a drug and its receptor at the molecular level [10–12]. Effective targeted drug delivery systems require four key requirements: retain, evade, target and release [13]. For formulations intended for i.v. administration, this means efficient drug loading into some type of delivery vehicle, sufficient residence in the circulation to reach intended sites of the body, retention by specific characteristics within intended sites (i.e., targeting), and drug release at the intended site within a time that allows for effective function of the drug. Obviously drug targeting to specific sites in the body requires different delivery systems depending on the drug delivery route selected. Here we will focus on drug targeting of i.v. administered formulations, and in particular formulations for delivering anticancer drugs.

3.1. Passive targeting and active targeting

Drug targeting strategies have frequently been divided into categories of "passive" and "active." These terms, however, do not represent what is really occurring *in vivo*, and tend to cause misunderstandings in defining a specific drug targeting strategy. So-called "passive targeting" is based on drug accumulation in the areas around the tumors with leaky vasculature; commonly referred to as the enhanced permeation and retention (EPR) effect (more on the EPR effect below). Passive targeting happens to almost all drug carriers whether such distribution is intended or not. While the EPR effect may be in effect for i.v. administered nanoparticles, the majority (>95%) of administered nanoparticles are known to accumulate in other organs, in particular the liver, spleen, and lungs. Does this mean there is passive targeting to these unintended organs? If >95% of an administered dose ends up at unintended sites of the body, the outcome can hardly be described as selective targeting. The bottom line is that "passive targeting" is a misnomer. Rather there is simply distribution of drug or drug delivery system by blood circulation. The term "passive targeting" needs to be replaced with "blood circulation and extravasation," which is not limited to drug delivery to tumors. Successful therapeutic application of "blood circulation and extravasation" can be achieved through technologies, such as locally activated delivery, where drug release and/or drug actions are limited to selective sites within the body such as a tumor but not the liver.

"Active targeting" is used to describe specific interactions between drug/drug carrier and the target cells, usually through specific ligand–receptor interactions [14–18]. Ligand–receptor interactions are possible only when the two components are in close proximity (<0.5 nm). The term "active targeting" has a flavor of guiding a drug/drug carrier to a target site like a cruise missile does. Current drug delivery systems, however, do not have the ability to guide themselves to a target. They reach the target area as a result of blood circulation and extravasation followed by intratumoral retention and distribution. The term "active targeting" simply means a specific "ligand–receptor type interaction" for intracellular localization which occurs only after blood circulation and extravasation. This is why increasing blood circulation time by PEGylation (i.e., modifying the surface of nanoparticles with poly(ethylene glycol)) and/or improving the EPR effect is expected to enhance delivery to the tumor site (see below in Section 4). Previous studies have also shown that the presence of the tumor-targeting ligand does not always result in increased accumulation of the nanoparticles in tumors [19–21], suggesting that "active targeting" does not automatically translate into effective delivery to the entire tumor.

3.2. Classification of targeted drug delivery

At present, targeted drug delivery can be classified into two broad areas: systemic targeting and intracellular targeting, as listed in Table 1. Systemic targeting, which is based on blood circulation and extravasation, can be further classified into ligand–receptor mediated and locally activated drug delivery. The latter can occur either by self-triggered drug release as a result of a signal specific at the site or by externally activating drug release from the carrier. Self-triggered targeting is based on the presence of specific enzymes or pH changes at the target site *in vivo*, while externally-activated targeting is based on external factors, such as light, temperature, magnetic field, and ultrasound [22–24].

Delivery of drug carriers to the target cells can be only a part of the whole story of successful drug targeting. Some drug carriers may have to gain access to and get inside the cytoplasm of a target cell in order to release the drug at the optimum rate for pharmacological effectiveness as in the case of gene therapy or treatment with siRNA. In that sense, intracellular targeting is as important as systemic targeting. Thus, understanding mechanisms of intracellular localization is critical in developing some drug delivery systems [25], and various experimental tools have been developed recently to study intracellular trafficking of the drug carriers [26–29]. An improved understanding of intracellular trafficking mechanisms will likely allow for effective targeting to specific locations within targeted cells required to optimize the efficacy of certain drugs.

4. Current understanding on drug targeting of i.v. administered systems

Our current understanding of drug targeting to tumors is based on a combination of several independent concepts, involving events associated with the EPR effect, nanoparticle properties and design, increased retention in the circulation due to PEGylation, and ligand–receptor type interactions, as shown in Fig. 1. Each of these concepts is briefly discussed below to clarify the potential confusion associated with each concept.

4.1. The EPR effect

The main tumor targeting principle of many drug delivery systems is based on the EPR effect of nanoparticles first proposed by Professor Hiroshi Maeda [30,31]. The key observation of the role played by EPR started with the enhanced antitumor effect of arterially infused high-molecular-weight antitumor agent SMANCS dissolved in lipid lymphographic agent (Ethiodol®) in man [32]. SMANCS is the styrene–maleic acid copolymer (SMA)-conjugated protein antitumor agent neocarzinostatin (NCS). NCS is a seven-stranded β-sandwich protein secreted by *Streptomyces neocarzinostaticus* with antitumoral activity, but with significant general cytotoxicity [33]. The molecular weight of SMANCS is around 16,000 g/mol (two SMA chains with molecular weight of 2000 each were grafted to one NCS of 12,000). Ethiodol is an injectable radio-opaque diagnostic agent containing 37% iodine combined with ethyl esters of fatty acids of poppy seed oil.

Table 1
Classification of the current targeted drug delivery processes.

1. Systemic targeting based on blood circulation and extravasation
   a. Ligand–receptor interaction mediated
   b. Locally-activated delivery
      i. Self-triggered release of the drug at the target cells
      ii. Externally-activated release of the drug at the target cells
2. Intracellular targeting
   a. Low-pH activation technologies that use default pathway delivery to lysosomes
   b. Mechanisms that avoid (default) lysosomal delivery

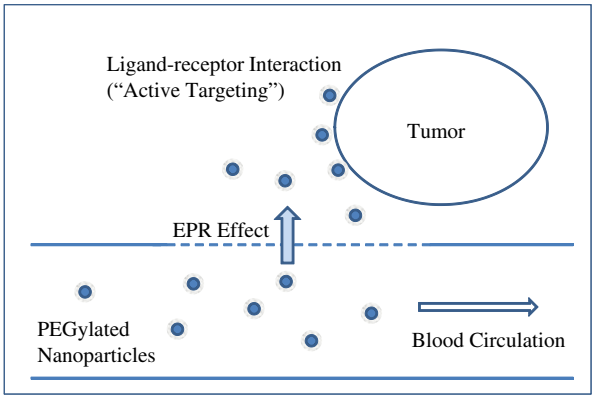

Fig. 1. Current factors on "targeted" delivery of nanoparticles to the tumor. Some of PEGylated nanoparticles circulating in blood accumulate at tumor region benefitted by the EPR effect. Specific ligand–receptor interactions can occur, if the nanoparticles possess a ligand to a specific marker on tumor cells.

The two major advantages of the SMANCS-Ethiodol formulation were selective accumulation of Ethiodol in tumor tissues, and more selective deposition of SMANCS in tumor tissues for longer periods of time relative to other conventional method at that time. About 90% of treated patients showed tumor size decreases of l0–99% of their original size. These pronounced effects of SMANCS were later attributed to the unique vascular characteristics of the tumor tissue and the lack of a lymphatic recovery system in the solid tumor [31]. Indeed, as clearly mentioned in one of Professor Maeda's ground-breaking papers [30], it was known that high-molecular-weight substances leak out of blood capillaries, but they can be recovered only through the lymphatic system, and that effective lymphatic function associated with tumors can be compromised [34]. Such a notion was verified by the SMANCS experiments.

While the more selective accumulation of SMANCS, as compared with the control or as compared with conventional delivery method, was apparent, the following points are often ignored in understanding the work by Professor Maeda [31]. The ratio of ^{51}Cr-labeled proteins detected in tumor and blood (the T/B ratio) was used to compare selective deposition of different proteins (NCS, SMANCS, ovomucoid, bovine serum albumin (BSA), mouse serum albumin, and mouse IgG (mIgG)) in tumor tissues. The time required to reach T/B ratios of 1/1 and 5/1 was 3.2 h and 19 h for SMANCS, clearly indicating the effective retention in tumors. Interestingly, the time required to reach these same 1/1 and 5/1 ratios was 9.3 h and 56.7 h for BSA, and 15.3 h and 72 h for mIgG. Both BSA and mIgG reached the same level of T/B distribution ratios, merely at a slower rate. While this article clearly mentioned the predominant accumulation of SMANCS in the liver, spleen, and tumor, tissue distribution studies clearly showed that ≤5% of administered SMANCS accumulated in the tumor during 72-hour observation with ≤1% of SMANCS remained in the blood after 24 h. The majority of the i.v. administered SMANCS went to the liver (42–50%) and spleen (24–40%). Accumulation in the liver and spleen was significantly reduced for BSA and mIgG, with higher deposition in tumor than SMANCS. The point here is that the more selective tumor deposition of SMANCS nanoparticles as compared with the control is one of the most important observations in cancer treatment, but at the same time, substantial accumulation in the liver and spleen must also be considered. Quite frequently, nanoparticles show higher tumor accumulation relative to controls, but only a very small fraction (<5%) of the total administered formulation is actually delivered to the intended target site.

4.2. Nanoparticle properties

In a landmark paper, Matsumura and Maeda showed that macromolecules in the molecular weight range of 15,000–70,000 g/mol, with certain additional properties, can effectively accumulate in a solid tumor [31]. This accumulation occurred faster with smaller molecules compared with larger, but the larger molecules were retained longer within the tumor. Thus, size is an important factor for controlling tumor accumulation kinetics and for preventing diffusion back into the systemic vascular bed. Other studies have shown that liposomes (90 nm diameter) extravasate from leaky tumor vessels but do not diffuse away effectively from the tumor even after a week [35]. The effect of nanoparticle size on blood circulation, however, is more complex and does not obey the same rules as observed for small molecule or protein-based chemotherapeutics.

The impact of particle size on biodistribution has been studied using particles with wide size distribution. Particle size is known to be intrinsically related to the rate of clearance from the blood circulation, and in general smaller particles (in the range of 50–300 nm) have slower removal from the circulation compared with those having larger size [36,37]. Since long-term circulation in blood is important for targeted drug delivery and sustained release [38], studies using nanoparticles with narrow size distribution will be critical for optimizing desired biodistribution parameters. Table 2 lists the size of particles and their presumed bioactivities in the body. Because of the heterogeneity of tumors and dynamic status of each tumor, it will be very difficult to assume any maximum single value for particles to exploit the EPR effect. The data in Table 2, however, collectively suggest that to be around 400 nm.

Nanoparticle stability is another important factor to consider for optimizing blood circulation and the ultimate accumulation within tumors. The size of drug-loaded polymeric micelles is commonly in the 100–150 nm range. While such formulations have been widely used, recent studies have shown that polymer micelles were not stable and dissociated in a matter of minutes to hours once in the

Table 2
Impact of particle size on biological systems.

Size	Biological systems and remarks
4.5 nm	Abundant small pores present in normal tissue endothelium [44]
25 nm	Relatively few large pores present in normal tissue endothelium [44]
20–50 nm	Average size of polymeric micelles without loaded drugs
100 nm	Frequently tested size of drug-loaded polymeric micelles [45].
150 nm	Proposed cutoff size for particle extravasation in liver [46]. Liver has blood vessels with fenestrations of 100–175 nm [47].
200 nm	Nanoparticles less than 200 nm have significantly longer circulation time due to low uptake by the reticuloendothelial system (RES) [48,49]
380 nm	A tumor-dependent functional pore cutoff size ranges from 200 nm to 1.2 μm, but the pore cutoff size of porous blood vessels in majority tumors is known to be 380–780 nm [50]. Thus, the range for the EPR effect should be similar.
400 nm	Sterically stabilized liposomes of 400 nm in diameter were able to penetrate into tumor interstitium [51]. Accumulation of hyaluronic acid-coated nanoparticles (400 nm) in the tumor tissue [52].
500 nm	The maximum size of nanoparticles allowing penetration through cell membranes is known to be 500 nm [53].
1 μm	Particles below 1 μm were taken up by Peyer's patches and then migrated to mesenteric lymph nodes [54].
5 μm	The upper limit for rigid particles circulating within the smallest capillaries [38].
40 μm	Particles larger than 40 μm have been used for embolization therapy [55].

systemic circulation [39–41]. Recent study has also shown that the polymer micelle systems tested released the encapsulated agent in a matter of only 15 min after i.v. infusion due to the interactions with blood components [42,43].

4.3. PEGylation

Accumulation of nanoparticles in tumors is based on blood circulation and extravasation, and extending time in the systemic circulation is a commonly strategy taken to increase the fraction of nanoparticles reaching a target tumor. Thus, it is assumed that nanoparticles with longer blood circulation times will have a higher probability of ending up within the tumor. To this end, PEGylation has been used extensively to modify the pharmacokinetics of the drug itself and/or the nanoparticles [56]. PEGylated nanoparticles have increased systemic circulation times, and this appears to be dependent upon the molecular weight of PEG used. When PEG of different molecular weights were i.v. injected into mice, it was found that the circulation half-life of PEG 6000 (molecular weight of 6000 g/mol) was only less than 30 min, while it was extended to a day with PEG 190,000 [57]. Poly(vinyl alcohol) (PVA) of different sizes also showed a similar trend in the systemic circulation time, which was increased from 90 min (PVA 14,800) to 23 h (PVA 434,000) [58]. Importantly, liver clearance is known to be enhanced with PEG having the molecular weight larger than 50,000 [57]. Although PEGylation is clearly beneficial, it is still not fully understood [59]. The effect of PEGylation depends on the molecular structure of PEG and the extent of PEGylation, but these parameters were seldom described in the literature.

While PEGylated nanoparticles have increased systemic circulation times, it is important to realize that the nanoparticle fraction entering tumors is still very limited. Usually only about 5% of the administered particles remains in the systemic circulation after 12 h with about 80% of the initial dose being eliminated in less than a few hours [16]. Several studies have shown a >100% increase in tumor accumulation of nanoparticles following PEGylation [16,60]. While this appears to be dramatic, the majority of i.v. administered nanoparticles still end up in the liver and spleen, with a lesser extent being in the lungs and kidneys. Additionally, >90% of these PEGylated nanoparticles are still removed from the systemic circulation within several hours. Only ~2% of the total i.v. administered dose is deposited in the tumor after 4 h of circulation. (A 100% increase resulted in an increase in total delivered dose from 1% to 2%). There is no doubt that PEGylation is a useful approach [59], but the question is whether it has been developed far enough to obtain clinically-relevant improvements for a targeted drug delivery strategy. The evidence seems to point to the contrary.

4.4. Ligand–receptor interactions ("active targeting")

As discussed above, the so-called "active targeting" refers to ligand–receptor type interaction after nanoparticles arrive at the target site via the systemic circulation and requires efficient interaction between the ligand and receptor after nanoparticles meet their target cell. Efficient ligand–receptor interaction for "active targeting" is dependent upon a variety of factors that include: the extent of target cell selective expression of the receptor relative to non-target cells, receptor availability on the target cell surface, the rate of internalization *vs* shedding of that surface receptor following ligand binding, etc. Further, the expression of a promising tumor-targeting receptor may not be homogenously distributed within a tumor or may change in its surface expression over time [61–63]. Thus, one can envisage conditions where the mere presence of a particle ligand on nanoparticles does not ensure "active targeting".

Cancer cell lines established and maintained *in vitro* using tissue culture that are used to identify promising receptors for "active targeting" may not represent the properties of primary cancer cells found in a patient's tumor. Further, it is simply not known what fraction of tumor cells express a specific receptor at any time point and what the expression level of that receptor in each individual cancer cells. Thus, promising outcomes from initial *in vitro* studies may be quite different from outcomes for xenograft studies using these established cancer cell lines in rodents and, more importantly, for the conditions present in spontaneous human tumors. Such complexities related to identifying an effective ligand–receptor interaction may help explain observations such as the lack of improved uptake of folate-targeted liposomes relative to non-targeted liposomes by tumor cells [64]. Here a question arises whether "active targeting" is really effective [65]. The issues of intratumoral distribution and cancer cell heterogeneity deserve more attention in targeted drug delivery. (See Section 6.2(Extravasation and intratumoral distribution) and Section 6.3(Tumor heterogeneity)).

5. Reality of tumor targeting

The concept of combining the EPR effect of nanoparticles with the longer systemic circulation properties that can be achieved following PEGylation has been explored. Frequently, antibodies or ligands intended to bind specific receptor molecules on tumor target cells have been PEGylated. Ligand-modified PEGylated nanoparticles showed increased drug accumulation at the target tumor site, but the actual percentage of PEGylated nanoparticles accumulating at the tumor site was only a few percent (at best) of the total i.v. administered dose. And yet, these ligand-modified PEGylated nanoparticles have been described as "magic bullets." While the ultimate goal of truly producing a "magic bullet" technology will require a number of small steps in that direction, no technology or combination of technologies has provided anything better than a few percent of the total administered dose reaching the intended target site. One could easily conclude wrongly that all administered nanoparticles go to the intended target site after prolonged circulation in the systemic circulation. It is not too uncommon to hear that nanoparticles only go to the target tumor cells and this is simply not true for technologies which have been described to date. Thus, the original "magic bullet" concept needs to be properly understood.

Paul Ehrlich's "magic bullet" concept has been translated to describe "drugs that go straight to their intended cell-structural targets," interacting only with the specific target molecule [12]. In reality, drugs never go straight to their intended targets. Drugs reach their targets as a result of properties that affect their stability in the systemic circulation, extravasation and intratumoral distribution, etc. In fact, any drug reaching the systemic circulation will be distributed throughout the body, and this is why the majority of drugs end up in various organs of the body, sometimes causing unacceptable side effects profiles. There has never been a drug or a drug delivery system that goes straight to the target in the body. Therefore, a more realistic interpretation of the "magic bullet" concept is for a compound that interacts with its target in an exclusive, highly specific fashion [12]. Of course, this interaction requires the two elements involved to be in close proximity. Because the magic bullet does not interact significantly with anything other than the target, the name "magic bullet" can be justified. But in reality, all drugs interact with more than one target, frequently with multiple targets resulting in side effects. (If a drug is chosen to interact with multiple targets by design, it is can be considered as a "magic shotgun" [66]).

In drug delivery, the "magic bullet" should refer to a system that delivers the majority, if not all, of a drug payload to the intended target without resulting in significant effects on non-target tissues. Based upon what have been presented in the scientific literature to date, we must conclude that we still have a long way to go to reach the "magic bullet" goal. The term "magic bullet" is most frequently discussed, however, in the context of cancer treatments. Due to the different mechanisms that could potentially be used to kill cancer

cells, the definition of "magic bullet" may be distinct for different approaches. In any case, at present, there are no drug delivery systems which meet the criteria of "magic bullets".

6. Factors to consider for effective tumor treatment

6.1. Clinical EPR effect

Chemotherapy is used after debulking surgery, aiming at unseen residual or metastasized malignant cancer cells. Targeted drug delivery using nanoparticles may provide an opportunity for treating tumors, particularly those which are large enough to develop vasculature or for patients who are not surgical candidates for debulking. In experimental animal models, the EPR effect has been shown to differ from tumor to tumor xenografts implanted at the same site [67], and from site to site following implantation of the same tumor [68]. Further, the tumor growth rate in mice is not comparable to that in human patients, and not much is known of blood vessel morphology in clinical tumors. The normalized accumulation of stealth liposome in clinical breast, head and neck and bronchus tumors varies from 2.7 to 53% ID/kg [69], reflecting the extremely heterogeneous nature of the EPR effect. Although there seems to be a clear EPR effect in clinical tumors, practical information on the extent of EPR effect of most solid tumors has not been available for the applications of nanoparticles. The clinical EPR effect could be influenced by numerous tumor biological factors. Overall, there is a definite need for systematic investigation of factors that could affect clinical EPR outcomes.

6.2. Extravasation and intratumoral distribution

A drug carrier, either in the form of soluble macromolecule or nanoparticle, can meet its target cell among various cell populations in a solid tumor after reaching the tumor vasculature. But the drug carrier has to extravasate through the openings in the blood vessels, and penetrate into and distribute within the tumor tissue. Translocation from the blood compartment to the tumor tissue is governed by convection and diffusion. The driving force behind convective flow is the pressure gradient. Unfortunately most solid tumors develop higher interstitial fluid pressure (IFP), ranging from 5 to 40 mm Hg depending on tumor size [70], compared with the normal tissue IFP which is typically <3 mm Hg. Drug carrier extravasation by convective fluid flow depends on the difference in pressure between tumor IFP and capillary hydrostatic pressure (10–30 mm Hg) and the difference in colloid osmotic (oncotic) pressures in both compartments [71].

Drug carriers move into tumor tissue compartments by random Brownian motion through openings between vascular endothelial cells (fenestrae) whose size can be sufficiently large for the passage of a wide range of nanoparticles. The rate of diffusional extravasation at a given concentration gradient across tumor vascular fenestrae can be controlled by nanoparticle parameters such as size, shape, surface properties, and concentration within the tumor blood vessel as well as and biological factors of the blood vessel such as the density, distribution, and size of fenestrae. Importantly, it has been noted that the distribution of leaky openings (fenestrae large enough to allow passage of nanoparticles) along a tumor blood vessel is heterogeneous.

Once a drug carrier passes through tumor vascular fenestrae its intratumoral distribution will be controlled by parameters such as size, surface charge, shape and interactions with extracellular matrix (ECM), soluble factors and cells. Stealth liposomes do not seem to penetrate deeply into tumor tissue, as shown by a study using a mouse dorsal window model; staying adjacent to blood vessels even 2 days after injection, not spreading beyond 50 μm from the vessel walls [35]. Another investigation has shown tumor penetration depth to be dependent on the particle size with traveling distance of test particles in a solid tumor again being extremely limited [72]. This observation predicts a low contact probability for some nanoparticle to reach a majority of target cells within the tumor. Intratumoral distribution becomes even worse for nanoparticles that can interact with ECM components and non-target cells in a tumor as this further reduces the nanoparticle mobility.

Another potential barrier issue for applications using nanoparticles is that they may be of sufficient size to physically block tumor vascular fenestrae, impeding their own entry and that of subsequent materials administered prior to their clearance from these sites. Such an outcome will certainly diminish the EPR effect. Quantitative analysis of the EPR effect of each dose would be important to understand dose dependency as well as any effects of sequential multi-doses on bioefficacy. Although systemic biodistribution and intracellular trafficking are of primary interest to most drug delivery researches, intratumoral distribution is another critical factor to consider in developing clinically useful targeted drug delivery systems.

6.3. Tumor heterogeneity

Clinically, tumors are classified as one of multiple potential subtypes using histological and genetic profiling. As an example, the tumor in the central nervous system can be categorized into one of 120 subtypes [73]. Most importantly for efforts related to targeted drug delivery to human disease, this singular categorization does not reflect the heterogeneous nature of intra-tumor cancer cells. While the existence of multiple cellular populations within a tumor has been understood since 1953 [74], research on the intra-tumoral heterogeneity in cancer cell population boomed in the early 1980s [75–79]. It is well-appreciated that, due to the mutable nature of cancer cells, their nature and characteristics can change both spatially and temporally with a tumor [80]. Accordingly, a variety of human cell lines can be derived from the same tumor. Naturally, *in vitro* and *in vivo* tests of targeted drug delivery systems with a selected cell lines may not properly represent genotype and phenotype characteristics of the primary tumor.

Both clonal evolution and cancer stem cell theories support intratumoral heterogeneity arising from genetic variation as well as epigenetic modification [81,82]. The cancer stem cell theory predicts the presence of hierarchical heterogeneity of cell populations as observed in blood cells derived from hematopoietic stem cells. It is straightforward to assume that long-lived somatic stem cells have higher probability of accumulating an oncogenic repertoire of genetic mutations than shorter-lived non-stem cells. Further, cancer stem cells are thought to be responsible for resistance to chemotherapy and radiotherapy, recurrence, and metastasis. Indeed, some have suggested cancer to be a sort of stem cell disorder. On the other hand, traditional stochastic clonal evolution theory suggests that any differentiated bulk cancer cells in a tumor can be transformed into cancer stem like cells (or tumor initiating cells) by genetic mutation and/or epigenetic variation in a random manner. Therefore, it is still unclear whether or not cancer is a stem cell disorder.

A tumor is not a monoculture or collective mass of a single cell type. Even a cultured cell line presents its heterogeneity in cell population including cancer stem-like cells with a different set of surface markers from other bulk cells which can survive from standard cytotoxic treatments [83]. It is important to note that surface markers defining a cancer stem-like cell population can be different from isolated cell lines from that tumor [84], reflecting the origin of cancer stem cells may not be identical. A more interesting feature of cancer cells is that they are in a dynamic state of the composition of tumor as reviewed in 1985 [80]. The dynamic nature of tumor composition is supported by genetic instability and epigenetic diversity of the cancer cells that is driven to adapt to new tumor microenvironment for survival by selection.

Epithelial–mesenchymal transition is one of the most known transformations of differentiated cells into more oncogenic phenotype that includes cancer stem-like cells in metastasis [85,86]. At any one time in a tumor there can be different subpopulations of several stages of transition between these different cell phenotypes in a type of dynamic equilibrium [87]. For instance, recent experimental observation supports that differentiated cancer cells (non-cancer stem cells) can revert to cancer stem-like cells by the actions of soluble factors such as interleukin-6 [87]. Tumors can thus be viewed as a community of various subpopulations that can respond to environmental cues induced by soluble factors and interaction with non-cancerous endothelial cells, pericytes, cancer associated fibroblasts, immune inflammatory cells, and stem and progenitor cells within the tumor stroma. These cells together can develop an extremely complex tumor microenvironment [88].

Taken together, aiming at cancer cells with a single surface marker results in aiming at a single population among mixed populations which are constantly changing and moving. Detection and diagnosis of a particular cancer cell type by a single surface marker can result in overestimation of cancers due to common shared feature of the given particular surface marker with normal cells within the tumor. Thus, single surface marker approaches are generally regarded as "outdated." The multiple surface marker approach is considered as a better option of cancer cell delineation and detection. There is significant research activity to explore new targeting moieties with advanced techniques, including phase display and aptamer screening approach using a primary tumor sample or a particular cell line where multiple surface markers are engaged. Such cell specific approaches are expected to result in targeting tumors with greater selectivity. While an exponential increase of research articles on cancer cell specific targeting of nanoparticles have been published for the last several decades [89], the approaches described have failed to translate into significant clinical advances. It was viewed as early as in 1987 that cell-specific targeted drug delivery is purely moot and may not be a feasible option to treat solid tumors in clinics [90]. Paul Erlich's "magic bullet" is still only a desirable concept.

6.4. Overexpression

"Overexpression" of a specific maker on the cell surface is one of the most widely used words in the literature to justify cell-specific targeted drug delivery. This term is only a relative description between target cells and non-target cells based on the differences in expression levels per unit cell mass (or tissue area). However, actual interactions occur by the absolute amount of interacting counter parts, not by a relative scale. According to the FDA guideline [91], when 10% of the cells in a breast cancer sample shows strong staining for HER2/neu, it is graded Score 3 and these patients are recommended for Herceptin® therapy. In a hypothetical case, if any particular receptor is 100 fold overexpressed on target cells, it can be 10% (target cell) *vs*. 0.1% (non-target cell). If the mass of non-target cells is more than 500-fold that of target cell mass, the amount of nanoparticles interacting with non-target cells would be 5 times more than that with target cells. (It is noted that unlike experimental animal tumors, the volume ratio of tumors in a patient to the whole distribution volume of the carrier is extremely low). Under uniform accessibility to individual cells, such as floating cancer cells mixed with healthy blood cells, only a very limited fraction of carriers, as calculated by the expression levels of target and non-target cells, can have the opportunity to interact with target cells. In reality, many organs including blood, liver, lung and spleen provide significantly greater accessibility to the carriers than solid tumors. The concept of using "overexpressed" marker on cancer cells may require re-examination if we are to truly achieve targeted drug delivery.

7. Future

True targeted drug delivery is still beyond our grasp, but it is probably the single most important property that drug delivery systems should acquire for treating cancers and certain other diseases where it will be important to place a drug selective at specific site of the body. The information necessary to achieve effective drug targeting may already exist, and we simply are not able to extract the answers from all information currently available. By understanding our current misunderstandings on targeted drug delivery we will be in a better position to discover the solutions for true drug targeting. The current concept of ligand-modified PEGylated nanoparticles as "magic bullet" needs to be modified. It simply presents an inaccurate picture of a very complicated problem.

For cancer therapies, the ideal targeted drug delivery system is the one that delivers the drug only to the target tumor. The reality, however, is far away from that ideal scenario. The amount of drug delivered to tumor targets is much less than 5% at most. Our efforts instead may have to be focused on how to better exploit this moderate amount of the drug delivered to the target tumor. As tumors may not be eradicated by just aiming at one target, it may also be necessary to simultaneously aim at multiple targets. Thus, it may be worthwhile to develop "magic shotgun" strategies that deliver multiple drugs, and/or deliver the drug to multiple targets.

It will be difficult to change our current approaches on targeted drug delivery overnight, as such changes will impact not only on the strategies selected but also on the methods to identify and test the success of these approaches. Furthermore, in the absence of clear rationale for identifying new approaches, changing, *per se*, does not necessarily lead to improved outcomes. But without dramatic changes in our current approaches, the targeted drug delivery research is likely to make few, if any, significant advances in the future. No matter the advances made in nanoparticle technologies, the delivery to target sites will be limited by blood circulation and extravasation. No matter how selective a ligand is to a target cancer cell marker, the ligand–receptor interaction occurs only after delivery by blood circulation and extravasation. The ligand–receptor interaction will be complicated by the fact that the "over-expressed" targeted surface markers selected will also be expressed on the surface of non-cancer cells that are likely to be grossly in excess of the cancer cell burden.

It is not that nanoparticles are not useful; they represent an exciting and promising advance in our armamentarium to effectively treat a wide range of diseases including cancer. Rather, nanoparticles alone are likely to be simply not enough, and over-dependence on these materials will be insufficient for significant clinical benefits to be achieved. Development of truly targeted drug delivery systems will require an improved understanding of multiple factors, regulation of distribution in the blood, dynamic aspects of tumor spatial and temporal heterogeneity, complexities of diffusional barriers in solid tumors, and uncertainties that still exist regarding the EPR effect. In addition, we may not want to rely solely on a single tumor-specific marker over-expressed on cancer cells for specific tumor-targeted delivery.

Current targeted drug delivery approaches are all prepared by scientifically sound rationale. The limited success of current nanoparticles is mainly due to the fact that these materials are constructed according to engineering and biochemical principles alone. While the known current nanoparticles can increase the blood circulation time and facilitate partitioning into tumors via the EPR effect, potentially improving their ability to interact with target cell receptors, these promising materials do address issues such as the dynamic changes of cancer cells and tumor heterogeneity. It is time to take these factors into account for developing better targeted drug delivery systems. Dynamic changes in cellular events cannot be described by mathematical equations yet, and thus, it is difficult to predict the cellular behavior or responses to drug delivery systems. This, however, should

not mean that we can ignore these important factors in the design of targeted drug delivery systems. Recognizing what we are missing is the first step toward moving in the right direction to solving the many problems that remain.

Acknowledgments

This study was supported in part by these grants: NIHCA101850, CA122356, and CA140348 (YHB), and NIH CA129287 and Showalter Research Trust Fund (KP). The authors greatly appreciate careful reading and invaluable comments by Professors Randy Mrsny and Alexander T. Florence.

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