Chapter 3
Development and Application of Anticancer Nanomedicine

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3.1 Introduction: Development of Nanomedicine

There is growing interest in integrating nanotechnology with medicine, creating the so-called nanomedicine for disease diagnosis and treatment with unprecedented precision and efficacy [1]. Nanomedicines are drug- or imaging agent-containing carriers or devices with size ranging from a few to several hundred nanometers [2]. Although the term nanomedicine emerged only recently [1, 3], nanotechnology has been employed in drug delivery for decades [4]. In principle, nanomedicines are designed to enable the delivery of small molecules or macromolecular therapeutics to achieve improved disease treatment by circumventing various physiological barriers. The physiological barriers may prohibit the efficient permeation of nanomedicines with undesired sizes and surface properties. Therefore, there have been significant efforts on controlled formulation of nanomedicines. The majority of current nanotechnology platforms for chemotherapy have involved repackaging of traditional anticancer agents into various forms of nanometer-sized delivery vehicles, such as monomeric polymer–drug conjugates with sizes typically 10 nm or less [2], polymeric nanoparticles [5] or self-assembled amphiphilic block-copolymer micelles [6] in a size range of 20–100 nm, or lipid [7] and polymeric vesicles [8] (also known as liposomes and polymersomes, respectively) with sizes between sub-100 nm to submicrometers.

Liposomes are by far the most successful nanomedicine platform, accounting for 30–40% of nanomedicines that have been approved by the U.S. Food and Drug Administration (FDA) for their usage in the clinic [9]. The report of the first liposomal drug delivery system dates back to the 1960s [5]. Long-circulating liposomes using so-called stealth technique appeared in the literature in the 1980s [10].

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Since then, in particular after the improvement of circulation profiles of liposome through the use of polyethylene glycol (PEG) [11], numerous liposomal nanomedicines have been developed and tested in the clinic, with a handful of them being approved by the FDA. For instance, Doxil®, a PEGylated liposomal doxorubicin, was approved by the FDA in 1995 for treating AIDS-associated Kaposi sarcoma, among other liposomes including Abelcet®, DaunoXome®, DepotDur®, and Ambisome® for treating cancer or other diseases [12]. Polymeric nanomedicine, a subfield of nanomedicine that involves the use of polymeric nanostructures as drug carriers, was first reported in the 1970s [13]. Since then, polymer-based nanomedicines have undergone many preclinical and clinical investigations. Abraxane®, a 130-nm paclitaxel/albumin polymeric nanoparticle, is one such example that has been approved by FDA as a second line treatment of breast cancer [14]. Currently, there are over three dozen nanomedicines approved for clinical use, and more are expected in the coming years [9, 15]. More than 50 companies are developing nanomedicine-based therapeutics or diagnostics for cancer therapy, 34 of which were established since 2006 [16].

The development of the abovementioned therapeutic nanomedicines has been mainly focused on targeting the primary tumors through the so-called enhanced permeation and retention (EPR) effect, a passive targeting mechanism that refers to the accumulation of nanomedicines in tumor tissue facilitated by the highly permeable nature of the tumor vasculature and poor lymphatic drainage of the interstitial fluid in the tumor [17]. The newer generation nanomedicines, however, place greater emphasis on novel strategies to bypass biological barriers at the systemic, tissue, and cellular levels and to locate and target metastatic lesions. New chemistries and fabrication technologies allow precise control of nanomedicine formulation, making it possible to evaluate nanomedicine with the variation of one parameter at a time (e.g., size, surface property, and shape), which provides insight into the fundamental understanding of the interplay of these parameters and the in vivo performance of the nanomedicines. Conjugation chemistry plays a vital role in controlling the incorporation of therapeutics or targeting ligands to nanomedicine. For instance, "click chemistry," a powerful conjugation approach conceived by Barry Sharpless, has become a highly recognized method in the field of nanomedicine.

3.2 In Vitro and In Vivo Studies of Nanomedicines

To achieve the accumulation of nanomedicines in tumor tissue, they must first overcome various systemic barriers, especially the clearance from the circulation system via phagocytic uptake and hepatic filtration. Nanomedicines are then expected to extravasate the tumor vasculature, penetrate the tumor microenvironment, and get internalized into the targeted cancer cells to allow cancer cells—even those situated distal to the tumor vessels—to be exposed to the anticancer agent with sufficiently high concentrations. The nanomedicines' size, shape, and surface property all have a significant impact on the efficiency of bypassing these physiological barriers.

Although the optimal size of nanomedicines for prolonged circulation half-life is still unclear, there is general consensus that their size should be controlled below 200 nm [3] because particles with size over 200 nm tend to induce undesired responses by the reticuloendothelial system (RES) and are quickly cleared from the circulation. Particles 150 nm or smaller may escape through the fenestration of the vascular endothelium and get cleared from the blood circulation. Particles with size less than 20 and 10 nm may be cleared through the lymph nodes and renal systems, respectively [3, 18]. Penetration of intravascularly administered nanomedicines into the tumor mass has been proven difficult because of the high interstitial fluid pressure and complex extracellular matrix of the tumor tissue [19]. Chilkoti and coworkers evaluated and demonstrated the molecular weight (size) dependency on the tumor penetration using dextran-based delivery vehicles [20]. They found that dextrans with low molecular weights (3.3–10 kDa) can efficiently penetrate and homogeneously distribute in the tumor tissue, but dextrans with higher molecular weights (40–70 kDa) were observed only ~15 μm away from the vessel wall, indicating their low penetration/permeation efficiency in the tumor tissue. Using a three-dimensional, multicellular spheroid of human cervical carcinoma cells that simulate a solid tumor, Pun et al. observed similar size dependency of nanoparticles on tumor penetration. Polystyrene nanoparticles with 20 or 40 nm sizes readily penetrated the simulated tumor and distributed homogeneously, whereas 100 and 200 nm particles showed restricted penetration. Interestingly, when nanomedicines were coated with extracellular matrix-disrupting collagenase, tumor penetration of the 20 and 40 nm particles was enhanced by roughly tenfold [21].

Geng et al. recently reported that the shape of delivery vehicles also has a significant effect on biodistribution [22]. They evaluated cylinder-shaped filomicelles (20–60 nm in cross-sectional diameter and a few micrometers in length) in rodents and found that the filomicelles could persist in the circulation up to 1 week after intravenous injection. The circulation half-life is about ten times longer than the half-life of their spherical counterparts, which is presumably caused by the fact that these cylinder-shaped delivery vehicles are more readily extended by flow forces and therefore are less likely to interact with and get taken up by the phagocytic cells. This interesting finding may shed light on the design of a new generation of drug delivery vehicles for enhanced circulation time and improved in vivo performance. A separate study using polymeric nanostructures with various shapes (e.g., cylinder and cube) also demonstrated the high impact of shape on the biological response of nanomedicine [23]. Cylindrical nanostructures with an aspect ratio (height/width) of 3, for example, can be internalized into cells four times faster than those with an aspect ratio of 2. It has yet to be determined whether these uniquely designed nanostructures could outperform the traditional, spherical nanoparticles in terms of biodistribution and antitumor efficacy.

Besides size and shape, surface characteristics and physical properties of nanomedicines can significantly influence the nanoparticle biodistribution. Positively charged particles are typically cleared much more quickly from the circulation than neutral or negatively charged particles [24]. The use of PEG to modify the surface of nanoparticles is critical to improve their circulation half-life and reduce the

plasma protein absorption to nanoparticles that could otherwise lead to opsonization, a process that involves surface deposition of blood opsonic factors (such as fibronectin) for enhanced recognition by macrophages [24]. There has been some progress made developing PEG-like, protein-resistant materials, exemplified by zwitterionic polymers [25], which exhibit high resistance to nonspecific protein absorption due in part to their neutral surface charge and hydrophilicity [26]. However, it is unclear at this time whether these materials could be viable, biocompatible alternatives to PEG. A recent study by Verma et al. showed that the surface pattern of nanomedicines can have a dramatic effect on their biological responses [27]. Gold nanoparticles coated with subnanometer striations of alternating anionic (sulfonate) and hydrophobic (methyl) groups can successfully penetrate plasma membrane without disrupting the membrane bilayer. This approach can be particularly useful for direct delivery of cargos to the cytoplasm. The surface-modified nanoparticles also showed improved resistance to protein absorption, providing another potential strategy for surface modification of nanomedicines.

3.3 Preparation of Nanomedicine with Controlled Properties

To develop nanomedicines with consistent in vitro and in vivo performance that can be utilized in targeted or personalized disease treatment, it is crucial to formulate these nanomedicines in a highly controlled manner. Conventional formulation strategies usually give rise to nanomedicines with heterogeneous sizes and predominantly spherical shapes. Particle Replication In Nonwetting Template (PRINT), a top-down nanofabrication technique developed by DeSimone and coworkers, addresses these limitations and allows the formulation of polymeric nanoparticles with precisely controlled sizes in various shapes other than spherical (e.g., cylindrical, cubic, discoid) using soft lithographic molding technology [28, 29]. The DeSimone group utilizes photocurable perfluoropolyether (PFPE) molds to emboss liquid precursor compounds, using highly fluorinated surfaces that are nonwetting to organic materials, which enables the fabrication of isolated objects with excellent control over shape and composition [29]. Another promising device-assisted nanomedicine formulation strategy was developed by Tseng [30, 31], and Karnik and Farokhzad [32–34], utilizing microfluidics to control rapid mixing of polymer and drug and to control droplet size to yield particles with uniform size.

Polymeric nanoparticles are usually prepared by coprecipitation of hydrophobic therapeutics with hydrophobic polymers, such as polylactide (PLA) or polylactide-*co*-glycolide (PLGA). The resulting nanoparticles typically have poorly controlled physicochemical properties such as low drug loading, undesired drug release kinetics, heterogeneous nanoparticle composition, and broad particle size distributions [5]. To address these challenges, a new drug-loading and formulation method was reported by Cheng and coworkers, using drug-initiated lactide polymerization followed by nanoprecipitation [35, 36]. In the presence of a metal catalyst (e.g., (BDI)Zn(II)N(TMS)$_2$ with BDI = 2-((2,6-diisopropylphenyl)amido)-4-((2,6-bisalkyl)

imino)-2-pentene), hydroxyl-containing drugs (e.g., paclitaxel, doxorubicin, or camptothecin) can quantitatively form metal-alkoxide complexes, which can subsequently initiate living, ring-opening polymerizations of lactide rings to form drug–PLA conjugates. Nanoprecipitation of the resulting drug–PLA conjugates gives rise to drug–PLA nanoparticles with controllable sizes between 50 and 150 nm and low polydispersity. These nanoparticles have high drug loading (as high as 40 wt%), high loading efficiency (97–100%), and controlled drug release kinetics without burst release effect. The bulky BDI chelating ligand on the metal catalyst also regulates the coordination of the metal catalyst only with the least sterically hindered hydroxyl group of the drug, providing additional control over the polymerization as well as the structure and composition of the polymer–drug conjugates. In a separate study to improve the formulation of nanomedicines via controlled chemistry, Shen and coworkers demonstrated a new concept by using drug molecules (e.g., camptothecin) to control the self-assembly of nanomedicines with minimal amount of carrier materials and therefore substantially enhanced drug loading [37]. Specifically, camptothecin was conjugated to an oligomer ethylene glycol (OEG), and the resulting camptothecin–OEG conjugate self-assembled into liposome-like nanocapsules via the hydrophobic interaction between camptothecin molecules.

Controlled conjugation chemistry is another tool playing a potentially vital role in controlling the incorporation of therapeutics or targeting ligands into nanomedicines. “Click chemistry,” a powerful conjugation technique conceived by Barry Sharpless, has become a highly recognized method in the field of nanomedicine, allowing conjugation of therapeutics or targeting ligands to nanomedicines with unprecedented site-specificity [38–40]. The click process involves 1,3-dipolar cycloaddition of an azide to an alkyne to form 1,2,3-triazole rings, a reaction known for its high efficiency and high specificity. Click chemistry proceeds well in aqueous solution [41] or even in live organisms [42, 43], and is independent of other functional groups [38], demonstrating excellent solvent and functionality tolerability. Click chemistry has been widely used lately in the synthesis of polymeric therapeutics, surface modification of nanomedicine, and bioconjugation for in vitro and in vivo applications [44, 45]. In one study, Wooley and coworkers developed a new methodology for the preparation of well-defined core-shell nanoparticles using click chemistry. An amphiphilic diblock copolymer (poly(acrylic acid)-*b*-poly(styrene)), partially functionalized throughout the corona with alkynyl groups, self-assembled in water into micelles and formed nanoparticles after click reaction between the alkynyl shell of the micelles and azide-terminated dendrimers as the cross-linking agent. The remaining azide termini of the dendrimer cross-linker were further utilized for a secondary click reaction to conjugate either fluorescence dye or therapeutics onto the nanoparticles’ surface [46]. Murphy et al. have recently demonstrated conjugation between azide-functionalized gold nanorods and an acetylene-functionalized enzyme (trypsin) through click chemistry. The click-conjugated enzyme showed substantially improved specificity and activity compared to the same enzyme linked to the gold nanorods by conventional bioconjugation chemistries [47]. Another innovative utilization of click chemistry was demonstrated by Bertozzi et al. in

noninvasive in vivo imaging in developing zebrafish [48]. They first treated zebrafish embryos with azide-containing, unnatural sugars to metabolically label their cell-surface glycans with azides. Subsequently, the embryos were treated with a difluorinated, cyclooctyne-containing fluorophore by means of copper-free click chemistry, enabling the visualization of glycans in vivo at subcellular resolution during the development of the zebrafish embryos.

3.4 Nanomedicine-Mediated Cancer Targeting

There have been enormous efforts of designing nanomedicines aiming for targeted delivery of therapeutics for improved treatment of cancer, cardiovascular diseases, and immunological diseases [49–51]. One of the key challenges is the design and formulation of clinically relevant, targeted nanomedicines [51]. Many nanomedicine platforms have been developed and used in targeted drug delivery applications, including dendrimers, liposomes, polymeric nanoparticles, micelles, protein nanoparticles, ceramic nanoparticles, viral nanoparticles, metallic nanoparticles, and carbon nanotubes [50]. To facilitate the clinical application of targeted nanomedicines, their formulation should involve the use of biocompatible materials and should be completed via simple, robust processes for the assembly of nanomedicine, incorporation of drug and targeting ligand, and purification, postformulation processing, large-scale preparation, sterilization, and storage. The formulation process should also allow facile optimization of physicochemical parameters of the targeted nanomedicines that can be critical to their PK/PD properties, cellular uptake behavior, and in vivo efficacy.

The FDA-approved nanomedicines for cancer therapy function mainly through the accumulation of nanomedicine in tumor tissues via the EPR effect in the leaky tumor vasculature [52] and the subsequent release of the payload to kill the cancer cells. This passive targeting process usually requires long-circulating delivery systems in order to achieve time-dependent accumulation in tumor tissue to substantially improve the biodistribution and pharmacokinetic profile of the therapeutic modality, compared to the conventional administration of unmodified drugs [53]. The efficiency of this passive targeting mechanism is largely determined by the physicochemical properties of the delivery system. Many liposomal or polymeric drug/protein nanomedicines were designed and developed mainly to address issues related to the pharmacological drawbacks of small molecule or protein therapeutics [2, 12, 54]. Without active targeting ligands, certain drug delivery systems with optimized biophysical and chemical properties can still exhibit tissue-specific accumulation [23, 27]. However, to further improve disease targeting, it is inevitable to integrate various active targeting strategies in nanomedicines through the incorporation of targeting ligands.

Targeted ligands can be either incorporated to formulated nanomedicines via surface conjugation or incorporated to prefunctionalized biomaterials prior to the nanomedicine formulation. The latter approach can simplify optimization and

potential scale-up of the targeted nanomedicine but can be very difficult to implement, especially in case of macromolecular targeting ligands (e.g., antibodies or aptamers) [35, 36, 55–58]. The majority of targeting ligand incorporation approaches still follow the former strategy. The conjugation of targeting ligands is one of the most critical steps in targeted nanomedicine formulation, which may result in decreased targeting efficiency due to poorly controlled ligand conjugation. Reasons are nonspecific binding prior to reaching the targeted disease tissue or anchoring on the targeted tissue surface too strongly, thus preventing homogeneous diffusion of the nanomedicine throughout the targeted tissue [59]. Therefore, optimization of the ligand density on the nanomedicine surface is a critical step to keep the subtle balance between anchoring affinity and tissue penetration, a key requirement for optimal therapeutic efficacy [60].

The proliferation of tumor cells requires sufficient nutrient supplies from blood. By stopping tumors from making new blood vessels, a process known as antiangiogenesis [61], not only the growth of solid tumors but also the tendency of tumor metastasis may be prohibited [62]. Over the last several decades, a handful of angiogenic targets have been explored in anticancer nanomedicine, which include the vascular endothelial growth factor receptors (VEGFRs), $\alpha_v\beta_3$ integrins, matrix metalloproteinase receptors (MMPs), and vascular cell adhesion molecule-1 (VCAM-1). Cell proliferation markers are another set of targets for cancer therapeutics, as many of these markers are significantly overexpressed on certain tumor cells. Actively targeting nanoparticles have followed the schemes of monoclonal antibodies to target cell proliferation receptors such as human epidermal receptors (HER) [63], transferrin receptors [64–66], and folate receptors [67].

Antibodies (Abs) are the most well-known targeting ligands used in targeted drug delivery. Over a dozen of monoclonal Abs have been approved by the FDA since 1997 [68], including Herceptin® (anti-HER2/neu) for breast cancer and Avastin (anti-VEGF-A) for metastatic colorectal cancer treatment. Hundreds of delivery systems based on Abs or their fragments are in preclinical and clinical investigations [69, 70]. As Abs are derived either from animals [71] or through phage display techniques [72], immunogenicity has always been a concern. The conjugation of Abs to nanomedicine is usually accomplished via coupling chemistry (e.g., carboxylate-to-amine or maleimide-to-thiol couplings). The drawback of this approach is the lack of conjugation site-specificity, which leads to substantially reduced targeting specificity and efficiency [73, 74]. Single-chain variable fragment (scFV) with high affinity to the targeted tumor tissue may restrict the localization and tumor penetration [75]. Another potential issue with the use of antibody-nanomedicine is the nonspecific binding to circulating free antigen or irrelevant receptors, which leads to reduced targeting efficiency [70]. Several strategies can be applied to address these concerns; one such strategy is to use affibody, the fragments of Abs, as the substituent of the high molecular weight Abs. Affibodies have comparable binding affinities and targeting efficiencies as Abs but have substantially reduced sizes (molecular weights of affibodies ~ 6kDa versus those of Abs ~150kDa) [76]; the latter is particularly important when they are used as the targeting ligands in nanomedicine. Engineered methods to increase the circulation time of antibodies have also been reported [77, 78].

Recent development in protein engineering may also facilitate the applications of Abs as targeting agents in nanomedicines [79, 80].

Aptamers (Apts) are single-stranded DNA or RNA that can fold into unique conformations. They can bind to specific targets, either small molecules or macromolecules, with very high affinity. Recently aptamers have been used as a new class of targeting ligands in nanomedicine-mediated cancer targeting and demonstrated great promise [81–84]. Aptamers are usually nonimmunogenic as they are developed via a combinational chemistry approach called systematic evolution of ligands by exponential enrichment (SELEX). As the synthesis of aptamers is achieved via an entirely chemical process, batch-to-batch variability can be substantially reduced. It is also possible to chemically modify aptamers by attaching fluorophores or functional groups for orthogonal bioconjugation; the latter approach holds significant advantage over Abs with respect to site-specific, controlled conjugation to nanomedicines. Aptamers exhibit remarkable stability over a wide range of pH, temperature, and organic solvents without loss of activity, and they can be modified to have improved stability against enzyme degradation, which is critical for their in vivo application. An additional advantage of using aptamers instead of antibodies as targeting ligand in nanomedicine is the potential of controlling the dosage of nanomedicines through the use of complementary DNA as the antidote [85, 86]. This option is particularly important in the case of an accidental overdose of a therapeutic nanomedicine that may otherwise cause significant, acute toxicity. The generalized manufacturing of antidotes to aptamers has recently been described [87]. One issue for using aptamers as targeting ligands for nanomedicines is that the number of available targets is still limited compared to the targets for antibodies. Identification of aptamers via an in vivo selection process has recently been reported, which may address this issue and open a new avenue to a large variety of potentially clinically relevant, tumor-specific aptamers [88].

Oligopeptides as targeting ligands can be selected through phage display. Oligopeptides are usually easy to synthesize and handle as compared to Abs or aptamers. Targeting mediated by oligopeptides, however, can be nonspecific. For instance, RGD (arginine–glycine–aspartic acid), one of the most well-known ligands with strong affinity to the cell adhesion integrin $\alpha_v\beta_3$ that is overexpressed in cancer cells, can target cancer and increase intracellular drug delivery in various preclinical tumor models [89, 90], but it also binds to other integrins such as $\alpha_5\beta_1$ and $\alpha_4\beta_1$. The nonspecific targeting and binding of RGD to other receptors might limit its potential in cancer-specific targeting [91, 92]. *Carbohydrates* in extracellular matrices (ECM) overexpressed in tumors, such as chondroitin sulfate [93] and hyaluronan (HA) receptor [94], allow them to serve as effective targets for cancer targeting. For example, HA coating of liposomes improved their circulation half-life and enhanced their targeting efficiency to HA receptor overexpressing tumors [94]. *Small organic molecule*-based cancer targeting ligands are much easier to prepare in large scale and to incorporate into nanomedicine as Abs, aptamers, or oligopeptides [95, 96]. A few examples such as folate and near-inferred fluorescent dye IR783 show interesting cancer targeting properties and may be promising ligands in nanomedicine-mediated cancer targeting [97, 98].

Tremendous effort has been undertaken to explore whether the incorporation of targeting ligands into nanomedicines can improve their in vivo biodistribution [99]. Early investigations using liposomes containing surface-conjugated, tumor-specific antibody showed that cancer targeting liposomes accumulated in the targeted tumor tissues twice as much as control liposomes [100]. Later, the work by Park and coworkers demonstrated that cancer targeting mediated by antibody–liposome conjugates had enhanced antitumor efficiency compared to control liposomes [101]. However, these antibody–liposome conjugates did not show improved accumulation in tumor tissues, rather the presence of antibody on liposomes improved their localization inside the target cancer cells [102]. Similar results showing improved accumulation inside the targeted cells rather than enhanced total tissue concentration were also reported by Davis and coworkers during their studies on transferrin-polymeric nanoparticle-mediated siRNA delivery [103]. Wittrup and coworker developed a mechanistic model to understand and predict the complex interplay between particle size, affinity, and tumor uptake [104]. Their model showed that particles with diameter of 50 nm or larger should have insignificant tumor uptake for both targeted and nontargeted groups, which is consistent with the observations by Park [102] and Davis [103]. Despite this size limitation, it is generally accepted that cellular uptake and efficacy of nanomedicines can be improved by the incorporation of targeting ligands [102, 103, 105]. Once nanoparticles extravasate into tumor tissue, their retention in the tissue and their uptake by cancer cells are facilitated by active targeting, followed by receptor-mediated endocytosis, both together resulting in higher intracellular drug concentration and increased efficacy [102, 105–107]. One additional aspect of vascular endothelial targeting for oncology or cardiovascular diseases using ligand-mediated active targeting is that the tissue accumulation of targeted nanomedicines is independent from the EPR effect [108]. Similar EPR independence was observed for immunological tissue targeting, utilizing targeted delivery systems as vaccines for active transportation from the lymphatic vessels to the draining lymph nodes, targeting the lymph node-residing dendritic cells [109].

Applying the optimal combination of drug delivery vehicles and suitable targeting ligands for specific disease, targeting may become clinically important. One example supporting this statement is the phase I clinical study of CALAA-01 using Calando Pharmaceutical's RONDEL nanoparticle delivery technology, which demonstrated an RNAi mechanism of action in cancer patients [110]. RONDEL nanoparticle delivery technology, developed by Davis and coworkers [110], is a transferrin-targeting, polymeric system for siRNA delivery for solid tumor therapy. Using multimodal in vivo imaging techniques, Davis and his team showed that nontargeting and transferrin-targeting polymeric nanoparticles have the identical distribution and tendency of accumulation in solid tumors, but the targeted particles led to more pronounced gene inhibition within cancer cells [103, 111]. The transferrin-targeting ligand is used to enhance the cellular uptake of the nanoparticles, rather than concentrating the nanoparticles in the tumor. Davis and coworkers further demonstrated that the presence of intracellularly localized nanoparticles is quantitatively correlated to the dose of the nanoparticles administered [107].

3.5 Current Status and Future Perspective

As the field of cancer nanotechnology further matures with an increasing number of nanotechnologies moving closer to clinical applications, there is plenty of room for continued efforts in developing new, nanometer-sized carriers for the prevention of disease progression and dissemination. To achieve personalized anticancer nanomedicine, there are still many obstacles to overcome. Formulations of nanomedicines with precisely controlled parameters (i.e., drug loading, size, and release kinetics) in large quantity are still challenging. Techniques that can be broadly utilized for the incorporation of therapeutics into a variety of polymers with all translational issues fully addressed are significantly lacking. Much information has been accumulated for the correlation of various physiochemical properties of nanomedicines (e.g., size, surface functional groups, and shape) with the systemic biodistribution, and long-circulating nanomedicines can be prepared for some specific systems. However, long-circulating nanomedicines may not exhibit maximized anticancer effects if these nanomedicines cannot homogeneously distribute in solid tumor tissues and internalize into the target cancer cells. In fact, drug delivery nanomedicines that can successfully penetrate the ECM of tumor tissues are rare. Developing polymeric nanomedicines that can penetrate certain biological barriers (e.g., the blood–brain barrier) is still a formidable task for drug delivery scientists and engineers. Cancer targeting by incorporating homing ligands to the surface of nanomedicines has been attempted for many years. However, formulation of nanomedicines containing protein-based targeting ligands (e.g., antibodies) is extremely difficult to control and may only be made on small scales. Incorporation of antibodies or aptamers into nanomedicines may result in improved in vivo efficacy, but meanwhile may also result in increased accumulation of nanomedicines in undesired organs such as liver or spleen that contain a large number of macrophages cells. Solid formulation of polymeric nanoparticles often resulted in aggregation during postformulation processing (e.g., lyophilization), which substantially reduced their clinical applicability. Although these challenges are difficult to address, synergistic integration of the efforts of chemists, materials scientists, chemical and biomedical engineers, and physicians may facilitate the development of anticancer nanomedicine at an unprecedented pace and may eventually make it possible to develop chemotherapy in time-, tissue-, and patient-specific manner.

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