TUMOR-TARGETED NANOPARTICLES: STATE-OF-THE-ART AND REMAINING CHALLENGES

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1.1 INTRODUCTION

Bringing a drug only to target tissues without spilling any molecule in unwanted places would be an ideal goal for any pharmacological therapies [1]. Owing to the dose-limiting side effects of chemotherapy, a number of drug delivery strategies have been developed in the context of cancer, where “targeted” therapy is most anticipated. Most tumor-targeted drug delivery systems are based on the fact that cancer cells express various molecular markers that are distinguished from those of normal cells. In particular, nanomedicines have received enormous attention in the past decades as a potential tool to increase the selectivity of chemotherapy and diagnosis, due to the small size conducive to circulation and the large surface area to volume ratio that facilitates surface functionalization. Several nanomedicine products have been launched in the market or in the clinical development stage as summarized in Table 1.1. Newer approaches are actively developed to increase target selectivity, although the number of targeted nanomedicines that reached the later phase of
<table>
<thead>
<tr>
<th>Type of Nanoparticles</th>
<th>Formulation</th>
<th>Drug</th>
<th>Brand Name (Company)</th>
<th>Indication(s)</th>
<th>Current Status and Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-based or liposomes</td>
<td>Lipid–drug complex</td>
<td>Amphotericin B</td>
<td>Abelcet (Sigma-Tau)</td>
<td>Antimicrobial</td>
<td>Market [3]</td>
</tr>
<tr>
<td></td>
<td>PEGylated liposome</td>
<td>Doxorubicin</td>
<td>Doxil and Caelyx (Janssen)</td>
<td>Breast cancer, ovarian cancer, multiple myeloma, Kaposi’s sarcoma</td>
<td>Market [4]</td>
</tr>
<tr>
<td></td>
<td>Non-PEGylated liposome</td>
<td>Doxorubicin</td>
<td>Myocet (Enzon)</td>
<td>Breast cancer</td>
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<tr>
<td></td>
<td>Non-PEGylated liposome</td>
<td>Daunorubicin</td>
<td>DaunoXome (Galen)</td>
<td>Kaposi’s sarcoma</td>
<td>Market [6]</td>
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<tr>
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<td>Non-PEGylated liposome</td>
<td>Cytarabine</td>
<td>DepoCyt (Sigma-Tau)</td>
<td>Lymphomatus meningitis, leukemia, glioblastoma</td>
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<td>Ambisome (Gilead)</td>
<td>Fungal infection, cryptococcal meningitis</td>
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<td>PEGylated liposome</td>
<td>Cisplatin</td>
<td>Lipoplatin (Regulon)</td>
<td>Various malignancies</td>
<td>Phase III, investigational drug [9–13]</td>
</tr>
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<td>Heat-activated PEGylated liposome</td>
<td>Doxorubicin</td>
<td>Thermodox (Celsius Corporation)</td>
<td>Hepatocellular carcinoma, recurrent chest wall breast cancer</td>
<td>Phase III [14]</td>
</tr>
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<td>Sphingomyelin-based liposomes</td>
<td>Vincristine sulfate (targeted, Optisome™, details not provided)</td>
<td>Marqibo™ (Talon Therapeutics)</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>NDA submitted [15,16]</td>
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<td>Albumin-bound particles</td>
<td>Albumin bound nanoparticle</td>
<td>Paclitaxel (Albumin)</td>
<td>Abraxane® (Astellas)</td>
<td>Breast cancer</td>
<td>Market [17,18]</td>
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<td>Polymer-drug/ protein/nucleic acid conjugates</td>
<td>PEG conjugated</td>
<td>L-Asparaginase</td>
<td>Oncaspar® (Sigma-Tau)</td>
<td>Acute lymphoblastic leukemia</td>
<td>Market [19]</td>
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<td>Poliglumex (poly-L-glutamic acid) conjugated</td>
<td>Paclitaxel</td>
<td>Opaxio™ or previously Xyotax (Cell Therapeutics Inc.)</td>
<td>Nonsmall cell lung cancer, ovarian cancer</td>
<td>Phase III [20–23]</td>
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<tr>
<td>Conjugated with uniquely engineered macromolecular polymer core using specialized linkers</td>
<td>Irinotecan</td>
<td>Etirinotecan Pegol (Nektar Therapeutics)</td>
<td>Breast cancer, ovarian cancer, colorectal cancer</td>
<td>Phase III [24]</td>
<td></td>
</tr>
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<tr>
<td>PEGylated anti-VEGF aptamer</td>
<td>Pegaptanib sodium (VEGF)</td>
<td>Macugen (Eyetech, Inc.)</td>
<td>Age-related macular degeneration</td>
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<tr>
<td>Polymeric micelles</td>
<td>Methoxy PEG-PLA copolymer micelle</td>
<td>Paclitaxel</td>
<td>Genexol-PM® (Samyang Co.)</td>
<td>Breast cancer, lung cancer, ovarian cancer</td>
<td>Phase II [27,28]</td>
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<tr>
<td>Nanocrystals</td>
<td>Nanocrystal drug particles</td>
<td>Aprepitant</td>
<td>Emend (Merck)</td>
<td>Antiemetic</td>
<td>Market [29]</td>
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<td>Nanocrystal drug particles</td>
<td>Sirolimus</td>
<td>Rapamune (Pfizer)</td>
<td>Immunosuppressive</td>
<td>Market [29]</td>
</tr>
<tr>
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<td>Nanocrystal drug particles</td>
<td>Fenofibrate</td>
<td>Tricor (Abbott)</td>
<td>Hypercholesterolemia or mixed dyslipidemia</td>
<td>Market [29]</td>
</tr>
<tr>
<td>Dendrimers</td>
<td>Nanocrystal drug particles</td>
<td>Megestrol acetate</td>
<td>Megace ES (BMS)</td>
<td>Synthetic progestin</td>
<td>Market [29]</td>
</tr>
<tr>
<td></td>
<td>Carbopel® gel (cross-linked acrylic acid)</td>
<td>SPL7013 (antimicrobial agent)</td>
<td>VivaGel (Starpharma, Dendritic Nanotechnologies Inc., United States)</td>
<td>Bacterial vaginosis</td>
<td>Market, Phase III [3,30]</td>
</tr>
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<td>Cyclodextrin-based nanoparticles</td>
<td>Transferrin receptor-targeted cyclodextrin NPs, RONDEL™</td>
<td>siRNA targeting the M2 subunit of ribonucleotide reductase</td>
<td>CALAA-01 (Calando Pharmaceuticals)</td>
<td>Inhibition of tumor growth</td>
<td>Phase I trial, [31,32]</td>
</tr>
<tr>
<td>Inorganic nanoparticles</td>
<td>Streptavidin covalently attached to quantum nanocrystals (QDot)</td>
<td>—</td>
<td>Qdot® 800 Streptavidin Conjugate (Invitrogen)</td>
<td>Imaging, diagnostics, detection of proteins, nucleic acids</td>
<td>Market [33,34]</td>
</tr>
<tr>
<td></td>
<td>Superparamagnetic iron oxide nanoparticles coated with carboxydextran</td>
<td>—</td>
<td>Resovist (Schering)</td>
<td>Hepatocellular carcinoma</td>
<td>Market [35]</td>
</tr>
<tr>
<td></td>
<td>Iron nanoparticles</td>
<td>—</td>
<td>Feridex (AMAG Pharmaceuticals)</td>
<td>Detection of liver lesions</td>
<td>Market [36]</td>
</tr>
</tbody>
</table>

Notes: The table was created through internet search, product websites, and previously published articles [37,38]. Antibody–drug conjugates are omitted, but some publications list them under nanomedicine [38].
clinical trials is relatively low considering the prevalent excitement and the investment made till date [2].

This chapter discusses the rationale of targeted nanomedicines, different approaches to achieve this goal, current challenges, and considerations for their future development. Here, nanomedicines refer to particulate materials with a diameter in nanometer scale, prepared with natural or synthetic polymers, lipids, or inorganic solids, in the form of polymer–drug conjugates, liposomes, polymeric nanoparticles (NPs) or micelles, dendrimers, nanotubes, nanocrystals, nanorods, nanoshells, or nanocages [2]. Nanomedicines used as a carrier of a drug are often called nanocarriers. Nanoparticles and nanocarriers are also interchangeably used. We will use the term “nanoparticles” to represent various types of nanocarriers in this chapter.

1.2 FUNCTIONS OF NANOPARTICLES

Fates of a drug entering circulation largely depend on the properties of the drug. Small hydrophilic drug molecules do not bind to proteins in the blood and are quickly eliminated from the body. Hydrophobic drugs are eliminated relatively slowly through kidneys due to plasma protein binding, metabolized in the liver to a hydrophilic metabolite, and then eliminated from the body. On the other hand, the disposition and clearance of drugs encapsulated in NPs depend relatively less on the drug itself than on the properties of NPs during circulation [39].

Systemically administered NPs should be stable enough to retain a drug during circulation and able to extravasate at the target tissues and release the drug in the target tissues and/or inside the target cells to exert an action. Advancement in the nanotechnology has made it possible to incorporate therapeutic drugs in various types of NPs. NPs have contributed to the delivery of poorly water-soluble drugs and improving the bioavailability of drugs with poor stability [2,40,41]. One of the major concerns in cancer chemotherapy is multidrug resistance as most anticancer drugs are substrates of drug efflux transporters such as P-glycoprotein. NPs can be designed to overcome the resistance by bypassing efflux transporters [2,42]. Most significantly, NPs have gained enormous interest as a way of increasing drug delivery to target tissues, in particular, tumors. A detailed discussion of the use of NPs in drug delivery is available in other review articles [2,43].

NPs can be combined with contrast or imaging agents to visualize pathological tissues and monitor the progression of diseases [44]. Liposomes, micelles, and dendrimers are used to carry paramagnetic ions of indium-111 (\(^{111}\text{In}\)), technetium (\(^{99m}\text{Tc}\)), manganese (Mn), and gadolinium (Gd). Semiconductor quantum dots or gold NPs have been widely explored for target-specific imaging as combined with various functionalization strategies [45–47].

1.3 TUMOR-TARGETED NANOPARTICLES

Several strategies have been employed to achieve tumor-targeted drug delivery based on NPs. We briefly introduce these targeting strategies before we discuss challenges in translating NPs to commercial products.
1.3.1 Passive Targeting

Delivering anticancer agents to the tumors is a multistep process. Drugs delivered into the bloodstream should reach intratumoral region from systemic circulation by crossing tumor vasculature so that drug molecules have access to the tumor cells and stroma. Many solid tumors are characterized by leaky vasculature and poor lymphatic drainage [43]. New vasculature in the tumor is formed to sustain the tumor growth and provide nutrients; however, the structure is usually abnormal and defective with holes ranging from 400 to 600 nm in diameter [48–50]. The defective structure of blood vessels surrounding tumors allows NPs to extravasate and accumulate in the tumor tissues. The impaired lymphatic drainage further helps retain NPs in the tumors. Therefore, NPs with a size less than the cutoff of holes in the vasculature have a selective advantage in reaching tumors. This phenomenon is referred to as the enhanced permeability and retention (EPR) effect [51–53].

For NPs to take advantage of the EPR effect, it is important that the NPs circulate for a long period of time. It is widely known that NPs with hydrophobic or charged surfaces are readily opsonized and taken up by the reticuloendothelial system (RES), which is responsible for clearing macromolecules or particles present in the blood before they reach the target tissues [54,55]. Therefore, most NPs are surface-modified with a hydrophilic and electrically neutral polymer such as polyethylene glycol (PEG) (PEGylated) [54,56,57]. The PEGylated surface is resistant to protein binding and thus avoids the recognition by the RES [55,58,59]. For long-term circulation and effective extravasation via leaky vasculature of tumors, ~50–300 nm is considered an optimal size [60,61].

1.3.2 Active Targeting

Active targeting strategy was developed to further enhance tumor accumulation of NPs beyond the level possible with long-circulating NPs. Here, NP surface is decorated with targeting ligands that can actively bind to cell receptors overexpressed on the tumor cells [2,49,62–65]. Frequently used targeting ligands include antibodies, peptides, nucleic acids, and small molecular weight ligands binding to specific receptors [2,65,66]. For example, NPs are conjugated with the monoclonal antibody teratuzumab or herceptin to target HER2 receptor on breast cancer cells [59,67]. A docetaxel-NP formulation containing a small molecule ligand which targets prostate-specific membrane antigen has recently entered a Phase I clinical trial [68].

The rationale of active targeting is that a specific interaction between targeting molecules and cell receptors will increase the amount of NPs retained in tumors. An example is shown in a biodistribution study of $^{111}$In-labeled polymeric micelle NPs [69]. Nontargeted NPs (25 nm) were rapidly cleared from plasma as compared to nontargeted NPs with a size of 60 nm, resulting in twofold less total tumor accumulation. On the other hand, another 25 nm NPs functionalized with epidermal growth factor achieved a greater tumor accumulation than nontargeted ones in mice with tumors overexpressing EGF-receptors, reaching the same level as that of 60 nm nontargeted NPs [69]. However, it should be borne in mind that the
contribution of a targeting ligand is predicated on the extravasation of NPs to the tumors; thus, the ability to survive circulation for a long period of time still remains one of the most important requirements in the active targeting strategy. For effective active targeting, the density of target receptors should be in range of $10^4$ or $10^5$ copies per cell [2,70]. In addition to the number of binding sites, binding affinity also plays an important role. Multivalency of ligands can further enhance the affinity for target cells [71]. Receptors in a cell are internalized via different pathways and go through a continuous process of recycling [72]. NPs with targeting ligands can be internalized via the receptor-mediated endocytosis pathways and release drugs inside the cells.

### 1.3.3 Target-Activated Systems

Target-activated systems refer to NPs that remain stable until they reach target tissues and are activated by the extracellular or intracellular cues [64,73]. The NPs are designed to circulate similar to passively targeted NPs and transform to a more cell-interactive form in response to conditions unique to tumor tissues or intracellular environments. These conditions include acidic pH or enzymes abundant in the tumor extracellular matrix (ECM).

#### 1.3.3.1 pH-Activated Systems

NPs responsive to acidic pH can be classified into two categories: one activated in the tumor ECM, which may have a pH as low as $\sim$6, and the other activated in the intracellular lysosomes, which acidifies to pH 4–5. Tumors exhibit a slightly acidic pH in the range 6.8–7.2 compared to the physiological pH of 7.4 [74]. This acidity is due to the accumulation of lactic acid, induced by the increased glycolysis of tumor cells [75]. The acidosis of tumor is further enhanced as tumors develop hypoxia, which induces or selects for hyperglycolic cells [76]. The pH-sensitive NPs are composed of polymers, which are protonated at acidic pHs. Upon protonation of the polymer, the NPs are disintegrated to release drugs or change the surface properties to enter cells [64,77].

Most NPs entering cells via receptor-mediated endocytosis pathways end up in endosomes, which undergo a maturation process into late endosomes and lysosomes [72]. The luminal pH in these vesicles is acidic, varying from pH 6 (endosomes), pH 5–6 (late endosomes), to pH 4.5 (lysosomes) [72]. NPs are designed to escape the vesicles and/or release the payload in response to the low pH. Polycationic polymers such as polyethyleneimine or fusogenic peptides can be used in the formulations [78]. On entering the cell, a drug should reach its final destination to produce an action, which can be a nucleus or other organelles such as mitochondria, lysosome, and endoplasmic reticulum. NP trafficking to the specific organelles may be achieved by the use of specific peptide sequences [47,79].

#### 1.3.3.2 Enzymatically Activated Systems

Enzymes overexpressed in the tumor microenvironment are used for activating the NPs at tumor sites. An example of such enzymes is matrix metalloproteinases (MMPs) [80,81], which play a critical role in the invasion of tumor cells and angiogenesis [82]. Several studies have employed
MMPs for removing the PEG protection of NP surface at the tumor sites [83–85]. In these approaches, PEG is linked to the NP surface via a peptide linker sensitive to MMPs. Recently, cathepsin B has been utilized to activate a nanoprobe in a tumor-specific manner [86]. Cathepsin B is a lysosomal cysteine protease that plays a role in tumor progression [87]. The nanoprobe consists of glycol chitosan conjugated with a cathepsin B substrate peptide, a near infrared fluorescent dye, and a dark quencher, which self-assemble into 280 nm NPs. Upon the entrance into tumor cells, the cathepsin B substrate peptide is cleaved by intracellular cathepsin B, allowing for dequenching of the dye and emission of the fluorescence signals [86].

1.4 REMAINING CHALLENGES IN THE DEVELOPMENT OF TUMOR-TARGETED NANOPARTICLES

Several NP products, such as liposomes and polymer–drug conjugates, are currently in the market or in the late stage of development process, improving therapeutic efficacy and safety profiles of chemotherapeutics [36,40]. A large number of targeted NPs have been developed in the last three decades in an attempt to further improve the efficacy of NP-based therapy. However, several challenges remain to be overcome for developing NPs that can attract commercial interest and achieve significant clinical outcomes. Current limitations and challenges in the advancement of nanomedicine have been thoroughly discussed in recent review articles [36,60,88–92].

1.4.1 NP Stability

Stability of circulating NPs matters in at least two contexts. NPs carrying a drug must survive the body’s natural ability to clear the NPs and should be able to retain a drug during circulation. The former requirement is largely addressed by surface modification with a “stealth” coating with polymers such as PEG. However, recent studies have identified potential disadvantages of PEG [73]. For example, PEG can interfere with NP–cell interactions [93] and endosomal escape of NPs [93] after the disposition of NPs. It is also suggested that PEGylated liposomes are implicated with immune responses, which lead to an accelerated blood clearance of the second dose liposomes [94,95]. Alternative polymers or surface protection strategies are actively explored [73].

Stable retention of a drug during circulation is another important challenge. Despite the thermodynamic stability in buffers, some polymeric micelles or matrix-type NPs have been shown to quickly disintegrate and/or leak the payload upon contact with various blood components [64,96–98]. Chen et al. demonstrated that polymeric micelles composed of PEG-poly(D,L-lactic acid) block-copolymer dissociated and released the encapsulated fluorescent dyes within 15 min after intravenous injection [96]. Another study showed that a drug and the carrier (liposomes) exhibited different pharmacokinetics in blood, indicating that the drug was escaping the carrier prematurely [99]. The instability of NPs in circulation is a significant (yet often neglected) problem in the development stage. Success of a new NP system
depends on the availability of a method that can reliably predict the stability of NPs in a biological environment.

1.4.2 Heterogeneous Tumor Vasculature

Tumor accumulation of NPs mainly depends on their passive extravasation via the leaky vasculature feeding the tumors. While the defective architecture of tumor vasculature is widely exploited by the NP-based drug delivery, it is often ignored that the tumor blood vessels are heterogeneous [100,101] and do not exhibit the same degree of leakiness [102]. The heterogeneity of the EPR effect is even greater when different tumor models are compared. A biodistribution study of $^{111}$In-labeled PEGylated liposomes in 17 patients with locally advanced cancers showed that the levels of tumor liposome uptake varied from 0.5% to 3.6% of injected dose (ID) at 72 h, which translated into 2.7–53% of ID/kg of tumor (ID/kg) [103]. This study also showed that the tumor uptake of liposomes varied significantly with the site of the primary tumors, from 5.3 ± 2.6% ID/kg in breast cancers, 18.3 ± 5.7% ID/kg in lung tumors, to 33.0 ± 15.8% ID/kg in head and neck cancers [103]. Therefore, NPs relying on the EPR effect alone are unlikely to achieve reliable and predictable clinical outcomes.

1.4.3 NP Distribution in Tumors

The lack of functional lymphatics and the high vascular permeability result in elevation of the pressure in the interstitial tissue of solid tumors [104]. Furthermore, tumors develop various conditions to increase the stiffness of tissue [105]. The high interstitial fluid pressure and the stiffness of the tissue interfere with migration of a drug in tumor, limiting its effect on the central region of the tumor, a potential source of tumor relapse and metastasis [106–108].

Drug delivery with NPs is more significantly influenced by this challenge because of the size and surface properties [105]. Goodman et al. predicted that movement of 100–200 nm NPs in tumor spheroids would be much restricted even with the treatment of an ECM-degrading enzyme [109]. It was also experimentally demonstrated that the intratumoral distribution of 60 nm polymeric micelles was limited and localized in proximity to the blood vessel [69]. The size restriction is particularly problematic in the treatment of tumors with hypovascular and hypopermeable properties such as pancreatic tumors [110]. In the case of NPs employing active targeting strategy, high affinity binding to the receptors can lead to the “binding site barrier effect” in solid tumors, where the NP–receptor interactions may prevent migration of NPs to the interior of tissues [2,69,111,112]. While an active targeting strategy has the potential to improve the delivery of drugs through the intracellular uptake of NPs, the effect may be limited to the periphery of tumors when the size and binding site barrier play a dominant role in intratumoral disposition of the NPs.

1.4.4 Regulatory Considerations

The unique properties of NPs enabled by the size and surface pose different safety issues than free drugs and larger dosage forms [113]. The U.S. Food and Drug
Administration’s Nanotechnology Task Force, formed in August 2006, solicited the agency’s action to develop a regulatory guidance for manufacturers and researchers [114]. However, much remains uncertain as to how their safety should be tested and how much time and cost will be required for the approval of NP products, especially those designed with multiple functionalities. Such uncertainties negatively influence the investors and development partners, when the new product brings about only mild improvement over existing products.

New drug products must pass the regulatory scrutiny that examines their safety, effectiveness, and potential hazards. Reproducibility and predictability of the product’s performance are the most important quality criteria under any test methods and models. Accordingly, it is important to ensure that NPs have consistent properties, such as size, charge, shape, and density of surface functional groups. Increasing complexity of a formulation increases the difficulty of quality control as well as the development cost. Therefore, the current effort to develop new types of NPs must undergo a careful cost–benefit analysis prior to a significant investment.

1.5 FUTURE PERSPECTIVES

Several recent studies attempt to address the challenges discussed so far [105]. For example, Maeda et al. proposed the use of drugs that artificially augment the EPR effect [102]. Angiotensin II was used to elevate systemic blood pressure [115], and nitroglycerin or other agents inducing nitric oxide production were used to facilitate vascular blood flow and permeability [116]. These approaches have shown the potential to overcome the heterogeneity of the EPR effect. To overcome the limitations in NP distribution in tumors, ECM-degrading enzymes such as hyaluronidase and collagenase were proposed as a potential aid to reduce the stiffness of ECM and the interstitial fluid pressure [109,117]. While ongoing studies are likely to make these challenges under control, additional issues await future efforts. Here we discuss the remaining homework for the advancement of nanomedicine.

1.5.1 In Vitro Models

Preclinical studies of anticancer drugs and NPs are first carried out in cell culture models. The cell culture model is a convenient and inexpensive way of screening the effectiveness of a drug or an NP formulation. However, it is not unusual that these drugs and NPs showing great efficacy in cell culture models are often proven much less effective in vivo. The lack of predictability is due in part to the fact that a single cell population grown in a two-dimensional (2D) layer barely reflects the nature of human tumors, which involves an intricate cross talk between different cell populations and three-dimensional (3D) organization of cells and ECM [118]. There is an increasing understanding of the significance of 3D architecture of tumors, cell populations, matrix composition and mechanics, and the gradients of signaling materials in modeling tumors in vitro. Accordingly, active efforts are made to build
more realistic in vitro tumor models [119–121]. Lessons learned from tissue engineering and regenerative medicine are urgently solicited in these efforts.

1.5.2 In Vivo Models

Owing to the relatively low cost and well-established protocols, mouse models with allograft or human xenograft tumors are widely used in the evaluation of in vivo performance of NPs. In these models, cancer cells are inoculated (typically subcutaneously) in immunodeficient mice, allowed to grow into visually identifiable tumors, and treated with a new formulation to examine the pharmacokinetics, biodistribution, and pharmacological effects. However, several cases show that the results of preclinical studies are poorly correlated with Phase III outcomes. For example, in a preclinical study with mice bearing C-26 colon carcinoma, PEGylated liposomes carrying doxorubicin achieved significant survival benefits than free doxorubicin, attributable to their accumulation in tumors [122]. On the other hand, the PEGylated liposomal doxorubicin was at best equivalent to free doxorubicin in clinical efficacy (progression-free survival and overall survival) in a randomized Phase III trial with metastatic breast cancer patients [123]. In another example, a macromolecular conjugate of PTX and poly(l-glutamic acid) (PTX poliglumex) demonstrated a prolonged circulation half-life and greater tumor uptake and antitumor activity as compared to Taxol (PTX solubilized with Cremophor EL) at the same dose in a mouse model with syngeneic ovarian carcinoma [124]. However, the developer officially withdrew its application for a marketing authorization of PTX poliglumex for the first-line treatment of lung cancer patients because of the lack of therapeutic advantages over the comparators and unexplained toxicity [125].

In explaining the gap between the results of xenograft models and clinical outcomes, several limitations of current animal models may be considered [118,126]. First, the size and growth rate of tumors in mice are not comparable to those of human patients. Human patients with tumors large enough to detect with visual inspection would be candidates for surgical debulking rather than chemotherapy. Metastatic or microscopic residual tumors would be more appropriate subjects of targeted chemotherapy, but not much is known about the vasculature structure of those tumors and the effectiveness of the EPR effect. Second, since many animal models employ allografts or human tumor xenografts, the use of immunodeficient mice is inevitable. The potential consequences of immune responses to NPs are underrated in these models. Third, when human xenografts are inoculated in mouse models, the tumors recruit substances to build ECM from mice as they grow. The potential impact of this artificial arrangement on the architecture of ECM, cell–ECM interactions, and tumor propagation is barely considered in the interpretation of preclinical studies. Fourth, even though a xenograft can represent important attributes of the original tumors, it is uncertain whether it captures the genetic and epigenetic variability of tumors in its entirety [118].

When preclinical studies in animal models are essential steps providing the rationale for clinical trials, successful development of NP products depends critically
on the availability of reliable animal models. At this point, it is yet unclear what would be the best animal model for predicting clinical outcomes of a drug product. One potential alternative to the subcutaneous xenograft is an orthotopic model, where a xenograft grown in proximity to the tissues or organs from which the tumor cell line was derived [118]. This model has advantages over the subcutaneous model as it provides an environment closer to a normal milieu of the tumor. One may also envision that genetically modified animals will have a promise in recapitulating the progression of human tumors and increasing the predictability of clinical outcomes.

1.5.3 New Targets

Although the current state-of-the-art targeting strategies have largely relied on specific interactions between cell surface markers and ligands, a number of studies have also found that the efficacy demonstrated in vitro or in vivo model does not translate at the clinical level. This has partly to do with the fact that a tumor is not a collective mass of a single-cell population expressing a consistent level of markers at all times. To the contrary, a tumor is highly heterogeneous and dynamic both in composition and genetic makeup. This makes it difficult to achieve a consistent outcome with an NP targeting a single type of molecular target [60,91]. Targeting multiple markers simultaneously is a conceivable alternative to the current targeting strategy. In addition, quiescent cancer cells or tumor-initiating cells are suggested as a potential target of drug delivery [127]. The tumor-initiating cells or cancer stem cells (CSCs), a subpopulation of cancer cells similar to stem cells, are believed to differentiate into new tumors when specific conditions are met. CSCs are known to be resistant to current radio- and chemotherapy, and some believe that the surviving CSCs are partly responsible for metastasis and recurrence of the disease [127]. Potential targets for NP-based drug delivery to CSCs include cellular markers such as CD44 and CD133 and hypoxic conditions that lead to the formation of CSC niches [127].

1.5.4 New Therapeutic Agents

While NPs have contributed to reducing toxic side effects of traditional chemotherapeutic drugs [123,128], their accumulation (and side effects) in the RES and bystander organs is currently inevitable. NPs may be a more useful tool for the delivery of alternative therapeutic agents, which are inherently specific to tumor cells (thus benign to normal tissues) but unstable in circulation or do not have an effective means to access target cells. Nucleic acid therapeutics such as small-interfering RNA, microRNA, or antisense oligonucleotides have proven effective in suppressing intrinsic drug resistance and survival of cancer cells, but their delivery is challenging. Various NPs are currently developed for the systemic delivery of these gene therapeutics [129], and one of them has entered a Phase I clinical trial in 2008 [130].
Acknowledgments

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REFERENCES


REFERENCES


