A REVIEW OF CURRENT NANOPARTICLE FOR THE DELIVERY OF CANCER THERAPEUTICS

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ABSTRACT

The objective of this review is to outline current major cancer targets for nanoparticle systems and give insight into the direction of the field. This review seeks to provide an overview of available nanoscale drug carriers by exploring the wide variety of developed nanostructures and the most commonly used moieties for targeted delivery. Additionally, the use of nanoscale carriers will be motivated by examining tumor physiology and the specific barriers present within both the tumor microenvironment and systemic delivery.

Key words: Nanoparticle, Cancer, Active targeting, Passive targeting.

INTRODUCTION

Cancer remains to be one of the leading causes of death worldwide. Over the past several decades significant advancements have been made in our fundamental understanding of cancer biology; which has in turn lead to better diagnostic and treatment methods. Despite these advancements, the overall mortality of cancer still remains high. A major reason for this is our inability to administer therapeutic agents selectively to the targeted sites without adverse effects on healthy tissue. Current therapeutic strategies for most cancers involve a combination of surgical resection, radiation therapy, and chemotherapy. These therapies are associated with significant morbidity and mortality primarily due to their non-specific effects on “normal” cells. The increase in efficacy of a therapeutic formulation is directly correlated to its ability to selectively target diseased tissue, overcome biological barriers, and “intelligently respond” to the disease environment to release therapeutic agents.

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Nanotechnology coupled with advanced sophisticated therapeutic agents offers the most potential for addressing these challenges (Caldorera-Moore M, Peppas NA, 2009). In recent years, outstanding progress has been made in using nanovectors, liposomal and polymer-mediated delivery strategies to (a) target drugs to tumor cells through surface ligands and (b) increase localized delivery by increasing serum residence time. Although these strategies have reduced systemic toxicity, significant improvement on delivery strategies is still necessary to increase patient compliance and reduce chemotherapy-related side effects in cancer patients. In this review, we will highlight some of the limitations of current clinical treatment methods for cancer while also exploring novel research in nanotechnology for the creation of better targeted treatment moieties that have the potential to serve as drug carriers that can selectively target cancer cells and provide controlled release of chemotherapeutics.

Chemotherapy and its limitations

Chemotherapeutic agents are, in the broadest sense, small drug like molecules that disrupt the normal functioning of a cell by inhibiting replication or inducing apoptosis. Due to their proficiency at provoking cytotoxic effects, chemotherapeutic agents have been almost
exclusively utilized in the treatment of cancer, where they exhibit the most deleterious effects to rapidly proliferating cells (Feng SS, Chien S, 2003). Prominent chemotherapeutic agents include paclitaxel, doxorubicin, daunorubicin, cisplatin, and docetaxel. Paclitaxel and docetaxel are both taxanes, components that function by stabilizing the microtubules and preventing mitosis from progressing from metaphase to anaphase (Rowinsky M, Eric K, 1997). Doxorubicin and daunorubicin belong to a class of chemotherapeutics known as the anthracyclines. These molecules are among the most effective drugs available, inducing the greatest degree of cytotoxicity and used to treat the widest variety of tumor types including aggressive lymphoma, breast cancer, and myeloblastic leukemia (Minotti et al., 2004). Doxorubicin has been shown to target the topoisomerase-II-DNA complex, disrupting the DNA and preventing cellular replication (Haley B, Frenkel E, 2008). Similarly, cisplatin, a platinum-compound, modifies cellular DNA which activates signaling pathways that triggers apoptosis (Boulikas T, Vougiouka M, 2003). The primary concern with utilizing the aforementioned chemotherapeutic agents is their inability to differentiate between healthy and tumor tissue (Maeda H, 2001). The drugs will attack all cells without discrimination, being particularly harmful to any rapidly proliferating cells in the body such as hair, intestinal epithelial cells, and bone marrow (Feng SS, Chien S, 2003). The most cytotoxic agents are the most effective but often result in severe side effects. Doxorubicin is widely considered to be best anti-cancer drug available today but results in side effects such as, nausea, fatigue, and extensive and often fatal cardiotoxicity (Minotti et al., 2004). Oncologists must, therefore, optimize the balance between the effectiveness of the drug and a patient’s ability to tolerate the accompanying side effects (Feng SS, Chien S, 2003). Nanoscale carrier systems designed to target specific disease conditions could be utilized to alleviate some, if not all, of these cytotoxic effects to healthy cells.

TUMOR PHYSIOLOGY

Tumor biology plays an important role in drug delivery. The growth, structure, and physiology of a tumor all impact the ability of nanoparticle drug carriers to be delivered successfully. Understanding which aspects of tumor biology are beneficial and which are detrimental to delivery leads to the development of more effective and efficient drug carriers.

Growth of tumors

A single cancerous cell surrounded by healthy tissue will replicate at a rate higher than the other cells, placing a strain on the nutrient supply and elimination of metabolic waste products. Once a small tumor mass has formed, the healthy tissue will not be able to compete with the cancer cells for the inadequate supply of nutrients from the bloodstream. Tumor cells will displace healthy cells until the tumor reaches a diffusion-limited maximal size. While tumor cells will typically not initiate apoptosis in a low nutrient environment, they do require the normal building blocks of cell function like oxygen, glucose and amino acids. The vasculature was designed to supply the now extinct healthy tissue that did not place as high a demand for nutrients due to its slower growth rate.

Tumor cells will therefore continue dividing because they do so without regard to nutrient supply but also many tumor cells will perish because the amount of nutrients is insufficient. The tumor cells at the outer edge of a mass have the best access to nutrients while cells on the inside die creating a necrotic core within tumors that rely on diffusion to deliver nutrients and eliminate waste products. In essence, a steady state tumor size forms, as the rate of proliferation is equal to the rate of cell death until a better connection with the circulatory system is created. This diffusion-limited maximal size of most tumors is around 2 mm (Grossfeld GD et al., 2002; Jones A, Harris AL, 1998). To grow beyond this size, the tumor must recruit the formation of blood vessels to provide the nutrients necessary to fuel its continued expansion. An illustration of tumor development from a single cell to a diffusion-limited tumor is shown in Fig. 1. It is thought that there could be numerous tumors at this diffusion-limited maximal size throughout the body. Until the tumor can gain that access to the circulation it will remain at this size and the process can take years.

The exact molecular mechanisms that initiate angiogenesis at a tumor site are not known and could be unique to site of origin but more information about what factors play a role in this process is being discovered. As more is known about the molecular mechanisms that stimulate angiogenesis, the factors involved present new therapeutic targets to prevent tumor development.

CURRENT NANOPARTICLE SYSTEMS FOR CANCER THERAPIES

There are a variety of nanoparticle systems currently being explored for cancer therapeutics (Haley B, Frenkel E, 2006). The material properties of each nanoparticle system have been developed to enhance delivery to the tumor. For example, hydrophilic surfaces can be used to provide the nanoparticles with stealth properties for longer circulation times and positively charged surfaces can enhance endocytosis. The types of nanoparticles currently used in research for cancer therapeutic applications include dendrimers (Kukowska-Latallo JF et al., 2005), liposomes (Bellocq NC et al., 2003), polymeric nanoparticles (Betancourt T et al., 2007), micelles (Sutton D et al., 2007), protein nanoparticles (Veronese FM, Pasut G, 2005), ceramic nanoparticles (Montet X et al., 2006), viral nanoparticles (Flenniken LO et al., 2005), metallic nanoparticles
Nanoparticles are particularly attractive for drug delivery due to their varied composition, structure, and surface characteristics (Liechty WB, Peppas NA, 2012). The vast array of nanoparticle compositions and structures allow the carriers to be fine-tuned for specific applications and targets. The most common architectures for targeted drug delivery applications include: liposomes, micelles, dendrimers, nanospheres, and nanocapsules. This section will highlight the benefits and detriments of these different nanoparticle systems for their use as drug delivery vehicles.

Liposomes are composed of amphiphilic molecules that are comprised of both polar and nonpolar components that self-assemble into colloidal particles (Fig. 2a). This self-assembly produces a spherical structure with the polar components of the molecule contacting the polar environment and the nonpolar components contacting the nonpolar environment (Lasic DD, 1998). The most common classification of liposomes is by the number of lipid bilayers present in the colloidal structure, with unilamellar liposomes containing one lipid bilayer and multilamellar liposomes containing multiple lipid bilayers. Due to their amphiphilic nature liposomes are capable of encapsulating both polar and nonpolar compounds for delivery (Haley B, Frenkel E, 2008). Liposomes are attractive for drug delivery applications for numerous reasons, including their resemblance to cell membranes in both structure and composition. Additionally, liposomes can be readily formed with nontoxic, nonimmunogenic, natural and biodegradable amphiphilic molecules. Liposomes by themselves tend to be slightly sterically unstable and are cleared rapidly from the bloodstream. For drug delivery applications, this behavior is remedied by functionalizing the liposomal surface with poly(ethylene glycol) tethers to impart increased steric stabilization (PEG discussed in greater detail later in this review). The surface of the liposome can also be modified with ligands for active targeting. A pegylated biodegradable liposome was used to encapsulate doxorubicin and became the first liposome-based treatment for cancer (Doxil).

Micelles The micelle is composed of amphiphilic molecules that self assemble into a structure with a hydrophobic core and a hydrophilic exterior (Fig. 2b). Micellar structure lends itself well to drug delivery applications for multiple reasons. Micelles typically have diameters of less than 100 nm, allowing them to participate in extravasation through the fenestrations in tumor vessels and limiting their uptake by the MPS/RES system. Their hydrophobic surface characteristics also shield them from immediate recognition and subsequently increase circulation time (Lavasanifar A et al., 2002). Hydrophobic drugs can be loaded into the core of the micellar structure and protected by the hydrophilic corona during transport to the tumor site (Kwon GS, Kataoka K, 1995).

Dendrimers Dendrimers are highly branched molecules that display a high degree of monodispersity and a well-defined structure (Hughes GA, 2005). They are stable and have surfaces that can be readily functionalized with targeting ligands and molecules such as folic acid. Drug molecules can be encapsulated in the dendrimer’s multifunctional core and protected by the extensive branching. Drug molecules, such as paclitaxel, can also be attached to the exterior of the dendrimer (Fig. 2c) (Majoros INJ et al., 2006).

Nanospheres and nanocapsules Nanospheres consist of a spherical polymeric matrix within which a drug is encapsulated (Fig. 2d). The drug is typically distributed evenly throughout this matrix and released into the environment via diffusion. The composition of the polymer matrix and its ability to imbibe fluids will determine how rapidly the drug will be released (Brigger IN et al., 2002; Ratner BD et al., 2002).
Nanocapsules are often referred to as reservoir systems as they contain the active ingredient in a core separated from the environment by a polymeric membrane (Fig. 2e). By saturating the core, the active ingredient can diffuse through the membrane with an approximately constant release rate (Ratner BD et al., 2004a). This release behavior is attractive for drug delivery applications.

The above nanoparticle systems have been widely explored for diffusion-driven drug release due to their large surface-to-volume ratios, which allow for drug release at feasible and clinically relevant time scales. There is a surge in the development of nanoparticle systems that do not rely solely on diffusion mechanisms for drug release. Instead, this new class of nanoparticle is able to respond to environmental, chemical, thermal, or biological triggers (Peppas NA et al., 2012). These ‘smart materials’ will release their therapeutic payload only when triggered. A more complete review on environmentally responsive carriers was recently published by Liechty et al., Although the diffusion-driven nanoparticles are unable to respond directly to their environment there are means by which these systems can target and accumulate in the tumor interstitium.

TARGETING
Passive targeting of tumors using nanoparticles

Nanoparticle systems exploit characteristics of tumor growth for the use of a passive form of targeting. The tumor becomes diffusion limited at a volume of 2 mm3 or above. This diffusion limitation impacts nutrition intake, waste excretion, and oxygen delivery. The tumor is able to overcome the diffusion limitation by increasing the surrounding vasculature in an event called angiogenesis (Jones A, Harris AL, 1998). A characteristic of angiogenesis is aberrant tortuosity and abnormalities in the basement membrane and the lack of pericytes lining endothelial cells (Baban DF, Seymour LW, 1998). The incomplete tumor vasculature results in leaky vessels with gap sizes of 100 nm to 2 μm depending upon the tumor type (Hobbs SK et al., 1998). In addition, interstitial pressure is higher at the center of tumors than at the periphery since tumors lack a well-defined lymphatic system. The increased internal pressure causes an outward convective interstitial fluid flow, which decreases drug diffusion to the center of the tumor. However, drugs and nanoparticles that gain interstitial access to the tumor have higher retention times than normal tissues. The combination of leaky vasculature and poor lymphatic drainage results in what is known as the Enhanced Permeation and Retention (EPR) effect. Nanoparticles smaller than the fenestrations can enter the interstitium and be entrapped in the tumor. Passive targeting also involves the use of other innate characteristics of the nanoparticle which can induce targeting to the tumor, such as charge. Cationic liposomes are found to bind by electrostatic interactions to negatively charged phospholipid headgroups preferentially expressed on tumor endothelial cells (Krasnici S et al., 2003; Kunstfeld R et al., 2003; Ran S et al., 2002; Thurston G et al., 1998).

Passive targeting can be achieved by modulating the size, shape, and surface characteristics of the nanoparticle drug carriers. However, there remain significant barriers to transport that often result in insufficient drug concentrations at the tumor site and, consequently, little therapeutic efficacy (Gu FX et al., 2007). Furthermore, passive targeting suffers from some of the same limitations of traditional chemotherapy such as an inability to actively distinguish healthy tissue from tumor tissue.

Active targeting of cancer

Active targeting involves the use of peripherally conjugated targeting moieties for enhanced delivery of nanoparticle systems, as seen in Fig. 1. Although antibody targeting is regarded as a promising strategy, some groups have reported that antibody targeting does not increase tumor localization, but instead increases internalization in animal models (Kirportin DB et al., 2000; Hatakeyama H et al., 2007). The targeting moieties are important to the mechanism of cellular uptake. Long circulation times will allow for effective transport of the nanoparticles to the tumor site through the EPR effect, and the targeting molecule can increase endocytosis of the nanoparticles. The internalization of nanoparticle drug delivery systems has shown an increased therapeutic effect (Drummond DC et al., 2000; Inuma H et al., 2002; Kobayashi T et al., 2007; Lopes de Menezes DE et al., 1998). If the nanoparticle attaches to vascular endothelial cells via a noninternalizing epitope, high local concentrations of the drug will be available on the outer surface of the target cell. Although this has a higher efficiency than free drug released into circulation, only a fraction of the released drug will be delivered to the target cell. In most cases, internalization of the nanoparticle is important for effective delivery of some anticancer drugs, especially in gene delivery, gene silencing, and other biotherapeutics (Atobe K et al., 2007). In this review, the cancer targets for current nanoparticle systems have been organized according to selected characteristics of tumor growth and metastasis. These targets are the neovasculature of angiogenesis, uncontrolled cell growth, and direct tumor targeting. There is large overlap between these divisions which reflects the heterogeneity of tumor biology and the large potential for multiple targeting schemes using the same ligand. This section will focus on the most widely utilized active targeting ligands for tumor therapy including folate, transferrin, aptamers, antibodies, and peptides.
**Folate**

Folate has been one of the most extensively utilized ligands for targeted drug delivery devices. The folate receptor (FR), or the high affinity membrane folate binding protein, binds the folate molecule with extremely high affinity (Kd ~ 10⁻⁹) (Hilgenbrink AR, Low PS, 2005). This receptor is also over-expressed in a variety of tumors such as ovarian carcinomas, choricarcinomas, meningeomas, uterine sarcomas, osteosarcomas, and non-Hodgkin’s lymphomas (Sudimack J, Lee RJ, 2000). Particles conjugated with folate or folic acid and bound to a folate receptor are internalized by the cell and introduced to the cytoplasm (Fig. 3a). The drug is then released by the nanoparticle in the cytoplasm of the tumor cell and proceed to interact with intracellular component (Stella B et al., 2000). One such folate conjugated nanoparticle is a folate receptor targeted biodegradable polymeric micelle loaded with doxorubicin developed by Yoo and colleagues. Micelles were created from a copolymer of poly(D,L-lactic-co-glycolic acid) (PLGA) and poly (ethylene glycol) (PEG). The PLGA allows the particle to biodegrade after delivery of its drug payload and the PEG increases the circulation time of the particles. Doxorubicin was conjugated via a chemical linkage to the PLGA while the folate was added to the PEG. The micelle (Fig. 3b) was tested for cytotoxicity and cardiotoxicity (a common side effect of DOX) compared to free DOX on folate-receptor-positive cell lines. It was determined that these particles exhibited increased cellular uptake, circulation time, and decreased cardiotoxicity. The decrease of cardiotoxicity indicates that the targeting moiety was able to differentiate between healthy and tumor tissue with greater specificity than untargeted DOX. Furthermore, the increased cytotoxicity and cellular uptake shows that the folate-receptor actively internalized the conjugated particle into the cytoplasm (Yoo HS, Park TG, 2004).

**Transferrin**

Transferrin is another receptor-ligand pair that has been utilized for tumor targeting applications. Transferrin is a membrane glycoprotein that functions with its receptor, TFR, to aid in uptake of iron by the cell (Ponka P, Lok CN, 1999). Much like folate, when transferrin binds to its receptor it initiates endocytosis and is internalized into the cellular cytoplasm. The transferrin receptor is overexpressed by as much as 10-fold on tumor cells making it an attractive option for targeted delivery of chemotherapeutics via nanoparticle carriers (Sahoo SK et al., 2004). Sahoo and colleagues have focused a great deal of attention on developing transferrin-conjugated paclitaxel-loaded nanoparticles. The nanoparticles were made using copolymerized PLGA and poly(vinyl alcohol) (PVA), both well-studied and defined materials for drug delivery. Transferrin was conjugated to the nanoparticle surface and loaded with paclitaxel. The conjugated and loaded nanoparticles were introduced to a human prostate cancer cell line. These particles were compared to a simple solution of paclitaxel and loaded particles without transferrin. The transferrin-conjugated particles exhibited a sustained release profile and a cellular uptake three times greater than the unconjugated nanoparticles. Furthermore, the conjugated NPs reduced cellular proliferation by 70%, while the unconjugated NPs only reduced it by 35%. The free paclitaxel, by comparison, only reduced proliferation by 20% (Sahoo SK et al., 2004).

**Aptamers**

Aptamers are short oligonucleotides of RNA or DNA that can fold into various conformations and engage in ligand binding. However, finding such sequences is akin to finding a needle in a haystack, with only one in 1010 random RNA sequences folding into a configuration able to participate in ligand binding (Weiner LM, Adams GP, 2000). SELEX, or systematic evolution of ligands by exponential amplification, is a process by which researchers can comb through vast populations of RNA and DNA sequences to find new aptamers to act as targeting ligands (Wilson DS, Szostak JW, 1999). Benefits of aptamers include their small size (~15 kD), lack of immunogenicity, and the potential to readily penetrate and target tumor cells. It has been shown that, much like folate and transferrin, aptamers result in increased targeting specificity and more efficient drug delivery to tumor cells.

**Antibodies (monoclonal antibodies)**

Like aptamers, antibodies attached to the surfaces of nanoparticles target specific antigens present on the cell membrane. The use of antibodies as targeting moieties has been extensively investigated over the past decade and has resulted in numerous available treatments. Unconjugated antibodies have been shown to have antitumor effects on lymphomas, breast cancers, non-Hodgkin’s lymphomas, colorectal cancers and chronic lymphocytic leukemias (Mehren MV et al., 2003). Antibody-based treatments function by recognizing specific antigens located on the surface of cancer cells. Once an antibody–antigen interaction occurs it can induce antitumor effects by multiple mechanisms including interfering with ligand-receptor binding or suppression of protein expression (Mehren MV et al., 2003).

**Peptides**

Peptides have also been proposed as a potential targeting moiety for delivering chemotherapeutics. Peptides, much like antibodies, can be used to disrupt ligand–receptor interactions on tumor cells and lead to cessation of cellular proliferation. They have the added benefit of being much less expensive and complex to
manufacture than antibodies. Screening of potential protein ligands is typically completed using a combinatorial phage library. This technique results in ligands that range from 10–15 amino acids in length and are able to selectively bind to tumor targets with high affinity (Brissette R et al., 2006). One such tumor target is the avb3 integrin, which is present at elevated levels on tumor cells and is an essential component of angiogenesis (Brooks PC et al., 1994). This integrin is recognized by the arginine–glycine–aspartic acid (RGD) peptidic sequence (Byrne JD et al., 2000). The affinity of the RGD sequence to the avb3 integrin has potential to be exploited for drug delivery devices. Nasongkla and colleagues have functionalized the surface of polymeric micelles with a cyclic peptide containing the RGD sequence to deliver doxorubicin to Kaposi’s sarcoma cells (Fig. 3c).

**Fig 1. Tumor development from initial carcinogenesis to diffusion limited maximal size.**

**Fig 2. Particle schematics**

(A) liposome, (B) micelle, (C) dendrimers functionalized with complexed (left) and encapsulated (right) drug molecules, (D) nanosphere, and (E) nanocapsule.

**Fig 3. Targeted particles**

(A) example of a folate receptor targeted particle. Liposome functionalized with PEG tethers to impart STEALTH characteristics and folate for tumor targeting, (B) folate-conjugated PLGA-PGA polymeric micelle loaded with encapsulated doxorubicin and (C) cRGD functionalized PCL–PEG polymeric micelle containing encapsulated doxorubicin (Nasongkla N et al., 2004).
CONCLUSION
Nanoparticles used as drug carriers for chemotherapeutic agents have the potential to drastically improve the way cancer is treated. Targeted therapy can reduce the extremely severe side effects those undergoing chemotherapy must endure. In addition, targeted therapy can push the boundaries of the therapeutic indices by ensuring that the cytotoxic levels of drug are only observed at the desired tumor site. A wide variety of nanoparticle structures and targeting ligands speaks to the promise of wide-scale use of targeted nanoparticle drug delivery carriers. Increasing the specificity of the carrier and optimizing drug loading and release are essential tasks to improve the quality of these devices. Targeted nanoparticle drug carriers have the potential to revolutionize cancer therapy and improve both the quality and duration of a patient’s life.

REFERENCES


