Significant progress has been made in the development of new agents against cancer and new delivery technologies. Proteomics and genomics continue to uncover molecular signatures that are unique to cancer. Yet, the major challenge remains in targeting and selectively killing cancer cells while affecting as few healthy cells as possible. Nanometer-sized particles have novel optical, electronic, and structural properties that are not available from either individual molecules or bulk solids. When linked with tumor-targeting moieties, such as tumor-specific ligands or monoclonal antibodies, these nanoparticles can be used to target cancer-specific receptors, tumor antigens (biomarkers), and tumor vasculatures with high affinity and precision.

Conventional cancer therapy and diagnostics involves the application of catheters, surgery, biopsy, chemotherapy, and radiation. Most current anticancer agents do not greatly differentiate between cancerous and normal cells. This leads to systemic toxicity and adverse effects. Consequently, the systemic application of these drugs often causes severe side effects in other tissues (e.g. bone marrow suppression, cardiomyopathy, and neurotoxicity), which greatly limits the maximal allowable dose of the drug. In addition, rapid elimination and widespread distribution into nontargeted organs and tissues requires the administration of a drug in large quantities, which is uneconomical and is often complicated because of nonspecific toxicity.

Nanotechnology could offer a less invasive alternative, enhancing the life expectancy and quality of life of the patient. The diameter of human cells spans 10-20 µm. The size of cell organelles ranges from a few nanometers to a few hundred nanometers. Nanoscale devices can readily interact with biomolecules on the cell surface and within the cells in a noninvasive manner, leaving the behavior and biochemical properties of those molecules intact. In their ‘mesoscopic’ size range of 10-100 nm in diameter, nanoparticles have more surface areas and functional groups that can be linked to multiple optical, radioisotopic, or magnetic diagnostic and therapeutic agents. When linked with tumor-targeting ligands such as monoclonal antibodies, these nanoparticles can be used to target tumor antigens (biomarkers), as well as tumor vasculatures with high affinity and specificity. In this
article, we discuss different targeting strategies for nanoscale drug delivery systems (see Scheme 1), and offer a perspective on cancer nanotherapy.

**Passive targeting**

Solid tumors have a diffusion-limited maximal size\(^1,2\) of about 2 mm\(^3\) and will remain at this size until angiogenesis occurs, thus granting them access to the circulation\(^3\). Rapid vascularization to serve fast-growing cancerous tissues inevitably leads to a leaky, defective architecture and impaired lymphatic drainage. This structure allows an enhanced permeation and retention (EPR) effect\(^4,5\) (first described by Matsumura et al.\(^6\)), as a result of which nanoparticles accumulate at the tumor site. For such a passive targeting mechanism to work, the size and surface properties of drug delivery nanoparticles must be controlled to avoid uptake by the reticuloendothelial system (RES)\(^7\). To maximize circulation times and targeting ability, the optimal size should be less than 100 nm in diameter and the surface should be hydrophilic to circumvent clearance by macrophages (large phagocytic cells of the RES). A hydrophilic nanoparticle surface safeguards against plasma protein adsorption, and can be achieved through hydrophilic polymer coating (e.g. by polyethylene glycol (PEG), poloxamines, poloxamers, and polysaccharides) or the use of branched or block copolymers\(^8,9\). The covalent linkage of amphiphilic copolymers (polylactic acid, polycaprolactone, and polycyanonacrylate) chemically coupled to PEG\(^9-11\) is generally preferred, as it avoids aggregation and ligand desorption when in contact with blood components\(^7\).

An alternative passive targeting strategy is to use the unique tumor environment in a scheme called tumor-activated...
Prodrug therapy. The drug is conjugated to a tumor-specific molecule and remains inactive until it reaches the target\textsuperscript{12} (Fig. 1). Overexpression of the matrix metalloproteinase (MMP), MMP-2, in melanoma has been shown in a number of preclinical as well as clinical investigations. Mansour et al.\textsuperscript{13} reported a water-soluble maleimide derivative of doxorubicin (DOX) incorporating an MMP-2-specific peptide sequence (Gly-Pro-Leu-Gly-Ile-Ala-Gly-Gln) that binds rapidly and selectively to the cysteine-34 position of circulating albumin. The albumin-doxorubicin conjugate is cleaved efficiently and specifically by MMP-2, releasing a doxorubicin tetrapeptide (Ile-Ala-Gly-Gln-DOX) and subsequently doxorubicin. pH and redox potential have also been explored as drug-release triggers at the tumor site\textsuperscript{14}.

Yet another passive targeting method is the direct local delivery of anticancer agents to tumors. This approach has the obvious advantage of excluding the drug from the systemic circulation. However, administration can be highly invasive, as it involves injections or surgical procedures. For some tumors that are difficult to access, such as lung cancers, the technique is nearly impossible to use.

Active targeting

Active targeting is usually achieved by conjugating to the nanoparticle a targeting component that provides preferential accumulation of nanoparticles in the tumor-bearing organ, in the tumor itself, individual cancer cells, intracellular organelles, or specific molecules in cancer cells. This approach is based on specific interactions such as lectin-carbohydrate, ligand-receptor, and antibody-antigen.

Lectin-carbohydrate is one of the classic examples for targeted drug delivery\textsuperscript{15}. Lectins are proteins of nonimmunological origin that are capable of recognizing and binding to glycoproteins expressed on cell surfaces. Lectin interactions with certain carbohydrates are very specific. Carbohydrate moieties can be used to target drug delivery systems to lectins (direct lectin targeting), and lectins can be used as targeting moieties to target cell surface carbohydrates (reverse lectin targeting). However, drug delivery systems based on lectin-carbohydrate have been developed mainly to target whole organs\textsuperscript{16}, which can harm normal cells. Therefore, in most cases, the targeting moiety is directed toward specific receptors or antigens expressed on the plasma membrane or elsewhere at the tumor site.

Multiple drug resistance (MDR), which is a major challenge in chemotherapy, often stems from the overexpression of the plasma membrane P-glycoprotein (Pgp)\textsuperscript{17,18}. In general, Pgp acts as an efflux pump to extrude positively charged xenobiotics – including some anticancer drugs – out of the cell. Many tumor cells are resistant to doxorubicin, which is a Pgp substrate. To overcome the resistance, poly(cyanocarylate) nanoparticles have been developed\textsuperscript{19,20}. Adsorption of the nanoparticles onto the plasma membrane and the subsequent release of doxorubicin leads to saturation of Pgp. Furthermore, the negatively charged degradation products of the polymer form an ion pair and neutralize the positive charge of doxorubicin\textsuperscript{19}, enhancing the diffusion of the drug across the plasma membrane. Blagosklonny proposed an approach to selectively kill resistant cancer cells that is based on a temporary increase in the resistance of sensitive cells against certain drugs by specific protectors, such as pharmacological inhibitors of apoptosis\textsuperscript{21}. These protectors are pumped out by MDR cells, while increasing the resistance in sensitive cells that do not have active drug efflux pumps. After applying a cytotoxic drug, sensitive cells are protected and survive the exposure, while unprotected MDR counterparts are killed. By abolishing dose-limiting side-effects of chemotherapy, this strategy might provide a means to selectively treat aggressive and resistant cancers. Tsuruo suggested that antibodies to P-glycoprotein overexpressed on multidrug resistant (MDR) cells could make an attractive targeting moiety\textsuperscript{22}.

The overexpression of receptors or antigens in many human cancers lends itself to efficient drug uptake via receptor-mediated endocytosis (cellular ingestion) – see Fig. 2.
Since glycoproteins cannot remove polymer-drug conjugates that have entered the cells via endocytosis\textsuperscript{23,24}, this active targeting mechanism provides an alternative route for overcoming MDR.

The cell surface receptor for folate is inaccessible from the circulation to healthy cells because of its location on the apical membrane of polarized epithelia, but is overexpressed on the surface of various cancers, including ovary, brain, kidney, breast, and lung malignancies. Surface plasmon resonance studies reveal that folate-conjugated PEGylated cyanoacrylate nanoparticles have a ten-fold higher affinity for the folate receptor than free folate\textsuperscript{11}. Folate receptors are often organized in clusters and bind preferably to the multivalent forms of the ligand. Furthermore, confocal microscopy demonstrated selective uptake and endocytosis of folate-conjugated nanoparticles by tumor cells that bear folate receptors. Interest in exploiting folate receptor targeting in cancer therapy and diagnosis has increased rapidly, as attested by many conjugated systems, including proteins, liposomes, imaging agents, and neutron activation compounds\textsuperscript{9,11,25-31}.

Tumor targeting by antibodies with engineered properties (Table 1) is in its infancy, but holds much promise. The monoclonal antibody (mAb) BR96 (anti-sialyl Lewis Y antigen), conjugated with doxorubicin, has proved highly efficacious in tumour xenograft studies\textsuperscript{32} but, unfortunately, has shown little or no efficacy in Phase II trials for metastatic breast cancer\textsuperscript{33} and advanced gastric adenocarcinoma\textsuperscript{34}, respectively. Moreover, dose-limiting gastrointestinal toxicities are observed in the breast cancer trial, because the immunoconjugate binds to antigen-positive normal cells in gastric mucosa, small intestine, and pancreas\textsuperscript{33}. Calicheamicins\textsuperscript{35-37} and maytansinoids\textsuperscript{38} are the most extensively evaluated of many small-molecule toxins that are used for direct antibody arming, but indirect arming has met with better success. For example, anti-ERBB2 immunoliposomes loaded with doxorubicin show greater antitumor activity than the free drug or the drug loaded in nontargeted liposomes in several tumor xenograft models\textsuperscript{39,40}. Moreover, the systemic toxicity of the immunoliposome-targeted doxorubicin was much less than that of free doxorubicin. Bispecific antibodies, which are non-natural antibodies with two different epitopes, have been used most widely for the delivery of immune effector cells and, to a lesser extent, for the delivery of radionuclides, drugs, and toxins to tumors\textsuperscript{41,42}.

Alternatively, tumor vasculatures can be targeted to allow targeted delivery to a wide range of tumor types. A number of angiogenesis inhibitors are undergoing clinical trials (Table 2). Antiangiogenic therapy prevents neovascularization (the proliferation of blood vessels in different tissues) by inhibiting proliferation, migration, and differentiation of endothelial cells\textsuperscript{43}. Vascular endothelial growth factor (VEGF) is expressed in many solid tumors. A potent angiogenesis-

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**Table 1 Antibodies in cancer therapeutics.**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Antibody target</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist activity</td>
<td>CD40, CD137</td>
<td>Various</td>
</tr>
<tr>
<td>Antagonist activity</td>
<td>CTLA4</td>
<td>MDX-010</td>
</tr>
<tr>
<td>Angiogenesis inhibition</td>
<td>VEGF</td>
<td>Avastin\textsuperscript{TM}</td>
</tr>
<tr>
<td>Antibody-dependent cell-mediated cytotoxicity</td>
<td>CD20</td>
<td>HuMax-CD20</td>
</tr>
<tr>
<td></td>
<td>HER-2/neu</td>
<td>Herceptin\textsuperscript{®}</td>
</tr>
<tr>
<td></td>
<td>EGF receptor</td>
<td>HuMax-EGFr</td>
</tr>
<tr>
<td></td>
<td>CD33</td>
<td>Mylotarg\textsuperscript{®}</td>
</tr>
<tr>
<td>Disruption signaling</td>
<td>HER-2/neu</td>
<td>Pertuzumab (2C4)</td>
</tr>
<tr>
<td>Complement-dependent cytotoxicity</td>
<td>CD20</td>
<td>Rituxan\textsuperscript{®}, HuMax-CD20</td>
</tr>
<tr>
<td>Blockage ligand binding</td>
<td>EGF receptor</td>
<td>Erbitux\textsuperscript{™}</td>
</tr>
</tbody>
</table>
stimulating protein, it also increases the permeability of tumor blood vessels. This leads to swelling of the tumor, ultimately hindering the ability of cancer cells to recruit blood supply through angiogenesis. VEGF has been used in liposomes and polymeric nanospheres to deliver angiostatin and endostatin. The α\textsubscript{v}β\textsubscript{3} integrin is one of the most specific biomarkers that can differentiate newly formed capillaries from their mature counterparts. Although all endothelial cells use integrin receptors to attach to the extraluminal submatrix, one unique receptor (α\textsubscript{v}β\textsubscript{3} integrin) is found on the luminal surface of the endothelial cell only during angiogenesis. High-affinity α\textsubscript{v}β\textsubscript{3} selective ligands, Arg-Gly-Asp (RGD), have been identified by phage display studies. The cyclic form, which contains a conformationally constrained RGD, has a higher binding affinity than the linear form. Doxorubicin-loaded PEG nanoparticles conjugated to cyclic RGD and paclitaxel-cyclic RGD nanoparticles have been reported recently.

Perspective

Nanotechnology is beginning to change the scale and methods of drug delivery. For decades, researchers have been developing new anticancer agents and new formulations for delivering existing and new agents.

More than 40% of active substances identified through combinatorial screening programs are poorly soluble in water. The conventional, and most current, formulations of such drugs are frequently plagued with problems such as poor and inconsistent bioavailability. The most widely used method for enhancing solubility is to generate a salt. For nonionizable compounds, micronization, soft-gel technology, cosolvents, surfactants, or complexing agents have been used. Since it is faster and more cost effective to redesign the molecule than to develop a new one, a broadly based technology applicable to poorly water-soluble drugs could have a tremendous impact.

Paclitaxel (Taxol\textsuperscript{TM}) is a microtubule-stabilizing agent that promotes tubulin polymerization, disrupting cell division and causing cell death. It displays neoplastic activity against primary epithelial ovarian carcinoma, breast, colon, and lung cancers. Because it is poorly soluble in aqueous solution, the formulation that is currently available is Chremophor EL (polyethoxylated castor oil) and ethanol. In a new formulation used in Abraxane (recently approved by the FDA to treat metastatic breast cancer), paclitaxel was conjugated

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**Table 2: Angiogenesis inhibitors undergoing clinical trial.**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Antiangiogenic drug</th>
<th>Target</th>
<th>Stage of clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies targeting VEGF-A</td>
<td>Avastin\textsuperscript{™}</td>
<td>VEGF-A</td>
<td>Phase I, II, III</td>
</tr>
<tr>
<td>Antibodies targeting VEGFR-2</td>
<td>VEGF-Trap</td>
<td>VEGF-A</td>
<td>Phase I</td>
</tr>
<tr>
<td>Receptor tyrosine kinase inhibitors</td>
<td>IMC-ICII</td>
<td>VEGFR-2</td>
<td>Phase I</td>
</tr>
<tr>
<td>Endothelial cell proliferation inhibitors</td>
<td>SUS416</td>
<td>VEGFR-2</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>SU6668</td>
<td>VEGFR-2, bFGFR, PDGFR</td>
<td>Phase I, II, III</td>
</tr>
<tr>
<td></td>
<td>ZD 6474</td>
<td>VEGFR-2, EGFR</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>ABT-510</td>
<td>Endothelial CD-36</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>Angiostatin</td>
<td>Various</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>Endostatin</td>
<td>Various</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>TNP-470</td>
<td>Methionine aminopeptidase, cyclin dependent kinase 2</td>
<td>Phase I</td>
</tr>
<tr>
<td>Integran activity inhibitors</td>
<td>Vitaxin</td>
<td>Integrin α\textsubscript{v}β\textsubscript{3}</td>
<td>Phase I</td>
</tr>
<tr>
<td>Vascular targeting agents</td>
<td>Medi-522</td>
<td>Integrin α\textsubscript{v}β\textsubscript{3}</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>ZD 6126</td>
<td>Endothelial tubulin</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>DMXAA</td>
<td>Endothelial tubulin</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

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Fig. 3: Self-assembled polymeric nanoparticles for both tumor targeting and therapeutic functions. Insert: delivery of the nanoparticle drugs by receptor-mediated endocytosis and controlled drug release inside the cytoplasm.
to albumin nanoparticles\textsuperscript{56}. In addition to circumventing side-effects of the highly toxic Chemorphor EL, which include hypersensitivity reactions and toxicity to kidney cells and nerve tissue (nephrotoxicity and neurotoxicity)\textsuperscript{55,57}, the system cleverly takes advantage of albumin receptors for nerve tissue (nephrotoxicity and neurotoxicity)\textsuperscript{55,57}, the system cleverly takes advantage of albumin receptors for improved drug delivery to cancer cells.

For specific targeting, the differences between cancerous cells and normal cells, which include uncontrolled proliferation, insensitivity to negative growth regulation, and antigrowth signals, angiogenesis and metastasis can be exploited. Thanks to recent advances in proteomics and genomics, there is a growing body of knowledge of unique cancer markers. They form the basis of complex interactions between bioconjugated nanoparticles and cancer cells. Carrier design and targeting strategies may vary according to the type, developmental stage, and location of cancer. There is much synergy between imaging and nanotechnology in biomedical applications. Many of the principles used to target delivery of drugs to cancers may also be applied to target imaging and diagnostic agents. The full in vivo potential of cancer nanotechnology in targeted drug delivery and imaging can be realized by using strategically engineered multifunctional nanoparticles (Figs. 3 and 4).

REFERENCES