Nanoparticle and targeted systems for cancer therapy

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Abstract

This review explores recent work directed towards more targeted treatment of cancer, whether through more specific anti-cancer agents or through methods of delivery. These areas include delivery by avoiding the reticuloendothelial system, utilizing the enhanced permeability and retention effect and tumor-specific targeting. Treatment opportunities using antibody-targeted therapies are summarized. The ability to treat cancer by targeting delivery through angiogenesis is also discussed and antiangiogenic drugs in clinical trials are presented. Delivery methods that specifically use nanoparticles are also highlighted, including both degradable and nondegradable polymers.
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1. Introduction

Current cancer therapy usually involves intrusive processes including application of catheters to allow chemotherapy, initial chemotherapy to shrink any cancer present, surgery to then remove the tumor(s) if possible, followed by more chemotherapy and radiation. The purpose of the chemotherapy and radiation is to kill the tumor cells as these cells are more susceptible to the actions of these drugs and methods because of their growth at a much faster rate than healthy cells, at least in adults. Research efforts to improve chemotherapy over the past 25 years have led to an improvement in patient survival but there is still a need for improvement. Current research areas include development of carriers to allow alternative dosing routes, new therapeutic targets such as blood vessels fueling tumor growth and targeted therapeutics that are more specific in their activity. Clinical trials have shown that patients are open to new therapeutic options and the goal of these new chemotherapeutics is to increase survival time and the quality of life for cancer patients.

In all cases, the effectiveness of the treatment is directly related to the treatment's ability to target and to kill the cancer cells while affecting as few healthy cells as possible. The degree of change in the patient's quality of life and eventual life expectancy is directly related to this targeting ability of the treatment. Most current cancer patients' only selectivity in their treatment is related to the inherent nature of the chemotherapeutic drugs to work on a particular type of cancer cell more intensely than on healthy cells. However, by administering bolus doses of these intense drugs systematically some side effects will always occur and sometimes are so intense that the patient must discontinue therapy before the drugs have a chance to eradicate the cancer [1]. Unfortunately, not all treatments,

even if carried through to the oncologists specifications, are effective in killing the cancer before the cancer kills the patient. The advances in treatment of cancer are progressing quickly both in terms of new agents against cancer and new ways of delivering both old and new agents. Hopefully this progress can move us away from near-toxic doses of non-specific agents. This review will primarily address new methods for delivering therapies, both old and new, with a focus on nanoparticle formulations and ones that specifically target tumors.

1.1. 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 blood stream. 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
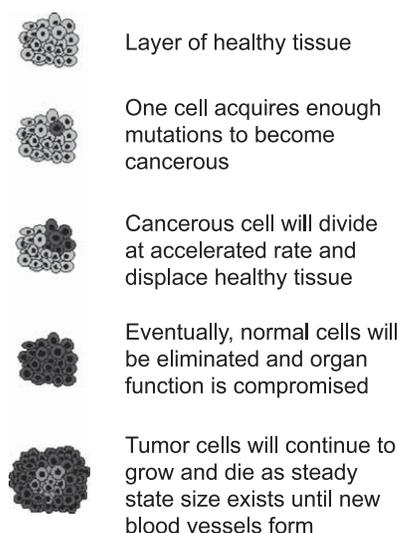
Fig. 1. Tumor development from initial carcinogenesis to diffusion-limited maximal size.

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^3 [2,3]. 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.

2. Targeted delivery

2.1. Achieving targeting by avoiding reticuloendothelial system (RES)

Nanoparticles will usually be taken up by the liver, spleen and other parts of the RES depending on their surface characteristics. Particles with more hydrophobic surfaces will preferentially be taken up by the liver, followed by the spleen and lungs [4]. Hydrophilic nanoparticles (35 nm diameter), such as those prepared from poly(vinyl pyrrolidone), show less than 1% uptake by the spleen and liver and 8 h after injection show 5–10% still circulating in the bloodstream. However, nanoparticles prepared of 50% PNVP and 50% *N*-isopropyl acrylamide (45 or 126 nm diameter) instead showed preferential uptake by the liver [5].

Particles with longer circulation times, and hence greater ability to target to the site of interest, should be 100 nm or less in diameter and have a hydrophilic surface in order to reduce clearance by macrophages [6]. Coatings of hydrophilic polymers can create a cloud of chains at the particle surface which will repel plasma proteins and work in this area began by adsorbing surfactants to the nanoparticles surface. Other routes include forming the particles from branched or block copolymers with hydrophilic and hydrophobic domains.

A recent review by Jain [7] describes experimental methods to ascertain the transport into solid tumors from the bloodstream. The heterogeneity of blood flow in non-necrotic regions of tumors is emphasized and the addition of even slower and unpredictable blood flow in necrotic and semi-necrotic regions only adds to the challenge of physically delivering treatment to cancerous tissues.

2.2. Targeted delivery through enhanced permeability and retention

A critical advantage in treating cancer with advanced, non-solution based therapies is the inherent leaky vasculature present serving cancerous tissues. The defective vascular architecture, created due to the rapid vascularization necessary to serve fast-growing cancers, coupled with poor lymphatic drainage allows an enhanced permeation and retention effect (EPR effect) [8,9]. The ability to target treatment to very specific cancer cells also uses a cancer's own structure in that many cancers overexpress particular antigens, even on their surface. This makes them ideal targets for drug delivery as long as the targets for a particular cancer cell type can be identified with confidence and are not expressed in significant quantities anywhere else in the body.

2.3. Tumor-specific targeting

Tumor-activated prodrug therapy uses the approach that a drug conjugated to a tumor-specific molecule will remain inactive until it reaches the tumor [10]. These systems would ideally be dependent on interactions with cells found specifically on the surface of cancerous cells and not the surface of healthy cells. Most linkers are usually peptidase-cleavable or acid labile but may not be stable enough in vivo to give desirable clinical outcomes. Limitations also exist due to the lower potency of some drugs after being linked to targeting moieties when the targeting portion is not cleaved correctly or at all. Recent research on an adriamycin-conjugated poly (ethylene glycol) linker with enzymatically cleavable peptide sequences (alanyl-valine, alanyl-proline, and glycyl-proline) has shown a greater selectivity to cleavage at tumor cells [11].

One such type of target is monoclonal antibodies which were first shown to be able to bind to specific tumor antigens in 1975 [12] but development of these antibodies into tools for cancer treatment took another 20 years. The ideal antigen should be expressed on all tumor cells but not expressed on critical host cells. There should be no mutation or variation and it should be required for cell survival or for a critical cellular function [13]. A number of targeted cancer treatments using antibodies for specific cancer types have been approved by the FDA and are summarized in Table 1 [13]. Many experts believe that these therapies using antibodies directed to cancer targets will dominate the market for the foreseeable future [14]. While these antibodies can prove to be therapeutic agents in their own right, they also have the ability to serve as carriers for drug delivery systems for even more effective and less intrusive cancer therapy.

These strategies both exploit the differences between a malignant cell and a normal cell. Some critical features include uncontrolled proliferation, insensitivity to negative growth regulation, angiogenesis, tissue invasion and metastasis, evasion of apoptosis (programmed cell death) and insensitivity to anti-growth signals [8].

2.4. Targeting through angiogenesis

Angiogenesis is a process vital to the continued development of a tumor mass. This process has been the subject of intense research due to its role in cancer development and has proven to be the result of numerous interactions between regulators, mediators and stimulatory molecules. These molecules regulate the proliferative and invasive activity of the endothelial cells that line blood vessels. Some of the most prominent angiogenesis stimulatory molecules include vascular endothelial growth factor (VEGF), basic fibroblast growth factor, platelet-derived growth factor and certain matrix metalloproteinases. Some endogenous angiogenesis inhibitors are the interferon family (α, β and γ), thrombospondin-1 and -2, certain tissue inhibitors of matrix metalloproteinases and protein fragments such as angiostatin and endostatin. A more extensive list of angiogenesis stimulatory and inhibitory factors is given in Appendices A and B.

Table 1
Currently available targeted cancer treatments using antibodies [13,14]

Generic name	Trade name	Manufacturer, year approved	Target and indication
Rituximab	Rituxan®	IDEC Pharmaceuticals, 1997	Anti-CD20 antibody or relapsed/refractory CD-20 positive B-call non-Hodgkin's lymphoma and low-grade or follicular-type lymphoma
Trastuzumab	Herceptin®	Genentech, 1998	Blocks HER2 receptor for HER-2 positive metastatic breast cancer
Gemtuzumabozogamicin	Mylotarg®	Wyeth Pharmaceuticals, 2000	Anti-CD33 antibody for relapsed/refractory acute myelogenous leukemia
Alemtuzumab	Campath®	Berlex Laboratories, 2001	Anti-CD52 antibody for B-call chronic lymphocytic leukemia
Ibritumomab tiuxetan	Zevalin®	IDEC Pharmaceuticals, 2002	Anti-CD20 antibody for Rituximab-failed non-Hodgkins lymphoma
Gefitinib	Iressa	AstraZeneca, 2003	Blocks epidermal growth factor receptors and tyrosine kinase activity for advanced non-small cell lung cancer

The formation of a new vessel from the pre-existing vasculature is characterized by a number of sequential events. The cellular events are virtually identical whether the stimulus results from the periodic neovascularization of the normal ovarian follicle or from a mass of tumor cells. Prior to neovascularization, endothelial cells exist in a near quiescent state with only about 1 in every 10,000 (0.01%) undergoing division at a given time [15]. The turnover rate of endothelial cells increases up to 50-fold during the formation of a new vascular sprout [16]. These events require the re-modeling of the extracellular matrix, which can also promote angiogenesis by unmasking angiostimulatory molecules. The extracellular matrix surrounds the vessels and contains motility-stimulating fragments and growth factors that combine to promote endothelial cell migration towards the tumor mass or other source of stimulus. These leading edge endothelial cells provide the framework for the new vessels [17,18]. Endothelial cell sprouts organize into tubular structures and connect to the vascular network.

Formation of new vessels during physiological angiogenesis is self-limited due to the production and release of angioinhibitory molecules. The equilibrium that normally exists between stimuli and inhibitors for angiogenesis is thought to be unbalanced during neovascularization initiated by tumor cells. Tumor cells are capable of secreting molecules that initiate the angiogenic process. The new vessels will allow the tumor to grow beyond the diffusion-limited maximal size. Some tumor masses never grow beyond this point, as they are incapable of recruiting new vessels. Acquisition of the angiostimulatory phenotype, also called the "angiogenic switch", is thought to result from a local imbalance between positive and negative regulators of angiogenesis.

The inability of the body to halt tumor-induced angiogenesis can have a number of explanations. Tumor cells in some cases no longer express angiogenesis inhibitors that would stop the process. Tumor cells and the surrounding stromal cells can be induced to express angiogenesis promoters at accelerated rates. As the blood vessels begin to form, immune cells that can secrete angiogenesis stimulators gain access to the tumor cells to continue to promote neovascularization. As the tumor cells are in closer proximity to blood vessels, tumor cells may disseminate from the tumor into the circulation. Upon finding a suitable environment, like a distant capillary bed or nearby lymph node, these cells can become metastatic foci of the primary tumor. The end result of a tumor that makes the angiogenic switch is a tumor capable of increased growth, fuelled by both paracrine and autocrine factors, with access to the blood stream to create additional tumors in other organs. The continued development of a tumor beyond the diffusion-limited maximal size is shown in Fig. 2.

Because a vascularized tumor is capable of increased growth and is more readily able to metastasize, increasing amounts of research are focused on developing treatments to slow angiogenesis and limit tumor growth and dissemination. Because so many
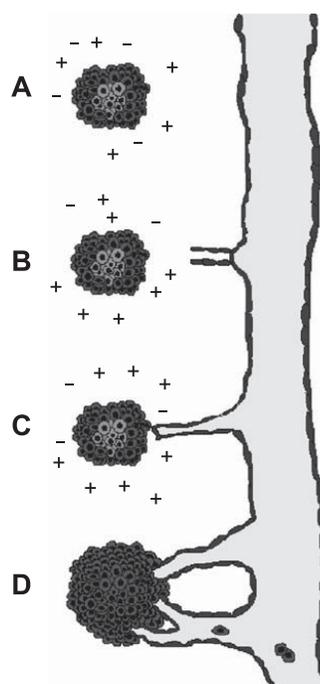
Fig. 2. Continued tumor development beyond diffusion-limited maximal size (A). In (B), the angiogenic switch has occured creating an imbalance of positive to negative regulators causing endothelial cell proliferation and migration. These endothelial cells form a vessel which extends towards the tumor and provides nutrients to sustain cell proliferation (C). A fully vascularized tumor (D) is capable of continued growth with metastatic potential due to the proximity to the blood stream.

different molecules are involved in angiogenesis there are many potential targets for therapy. Some examples of therapeutic strategies include limiting endothelial proliferation and motility, increased expression of angiogenesis inhibitors and use of molecules such as soluble VEGF receptor to try and decrease the amount of angiogenesis stimulatory factors at the tumor site. A comprehensive list of anti-angiogenic therapies in clinical trials can be found at http://cancernet.nci.nih.gov.

2.5. Targeting tumor vasculature

Targeting the tumor vasculature is a strategy that can allow targeted delivery to a wide range of tumor types [19–21]. The opportunities for this type of treatment were first discussed by Judah Folkman in 1989 [22]. The first vascular targeting was approved by the FDA in 1999 for treatment of age-related macular degeneration. In 2003, clinical trials with the antiangiogenic drug Avastin® (Genentech) showed that its use can prolong survival in patients with metastatic colorectal cancer. Avastin targets vascular endothelial growth factor (VEGF) which is a powerful angiogenesis stimulating protein that also causes tumor blood vessels to become more permeable. This permeability leads to swelling of the tumor and stops the ability of cancer cells to recruit a blood supply through the process of angiogenesis. VEGF has been shown to be expressed in many solid tumors including those of the lung, kidney, breast, ovary and gastro-intestinal tract [19]. Recent work in gene therapy has also worked to utilize VEGF and targets for the angiogenic inhibitors angiostatin and endostatin including delivery with liposomes and poly(N-vinyl-2-pyrrolidone) [20,21].

Recent work has also shown that gene delivery may also be targeted to neovasculature by coupling lipid-based cationic nanoparticles to an integrin αvβ-targeting ligand in tumor-bearing mice [23]. This study delivered a mutant Raf gene which blocks endothelial signaling and angiogenesis in response to multiple growth factors. This study compared, among other results, the gene expression in the tumor, lung, liver and heart for non-targeted particles, targeted particles and targeted particles injected with excess soluble targeting ligand. For non-targeted particles, limited expression (<0.5 ng/g tissue) was found in the tumor, lung and heart. For targeted particles significant expression was found in the tumor (4 ng/g tissue) and no expression in the lung, liver or heart. Perhaps most significantly, no gene expression was detected for targeted particles injected with an excess of soluble ligand which leads to the conclusion that the ligand was selectively bound to the tumor over the ligand-nanoparticle combination and fully blocked the particles from reaching the tumor. The tumor size was also noted with treatment begun at day 9 of tumor growth. All mice treated with PBS control, empty targeting nanoparticles or loaded targeting nanoparticles injected with an excess of targeting ligand showed no signs of a slowing of tumor growth and had to be killed by day 25 because of the growth in size of the tumor. However, those mice injected with the gene-loaded targeting nanoparticles showed a significant regression in tumor size, with four of the six mice showing no tumor and the others with >95% reduction in tumor mass and >75% suppression in blood vessel density. This tumor regression was sustained for >250 days.

A detailed list of antiangiogenic agents currently under study may be found at the web site for the Angiogenesis Foundation (http://www.angio.org) and are summarized in Table 2.

Table 2
Antiangiogenic drugs currently in clinical trails for cancer

2ME2	EMD 121974	Photopoint
Angiostatin	Endostatin	PI-88
Angiozyme	Flavopiridol	Prinomastat (AG-3340)
Anti-VEGF RhuMAb	Genistein (GCP)	PTK787 (ZK22584)
Apra (CT-2584)	Green Tea Extract	RO317453
Avicine	IM-862	Solimastat
Benefin	ImmTher	Squalamine
BMS275291	Interferon alpha	SU 101
Carboxyamidotriazole	Interleukin-12	SU 5416
CC4047	Iressa (ZD1839)	SU 6668
CC5013	Marimastat	Suradista (FCE 26644)
CC7085	Metastat (Col-3)	Suramin (Metaret)
CDC801	Neovastat	Tetrathiomolybdate
CGP-41251 (PKC 412)	Octreotide	Thalidomide
CM101	Paclitaxel	TNP-470
Combretastatin A-4 Prodrug	Penicillamine Photofrin	Vitaxin

3. Delivery of specific agents

3.1. Paclitaxel

Paclitaxel is a microtubule-stabilizing agent which promotes polymerization of tubululin causing cell death by disrupting the dynamics necessary for cell division. It has neoplastic activity especially against primary epithelial ovarian carcinoma, breast, colon, and non-small cell lung cancers. Paclitaxel is poorly soluble in aqueous solutions but soluble in many organic solvents such as alcohols. It therefore lends itself well to more advanced formulation strategies. The currently available formulation includes Chremophor EL (polyethoxylated castor oil) and ethanol for solubilization however Chremophor EL is toxic and shows side effects such as hypersensitivity reactions, nephrotoxicity and neurotoxicity [24]. Formulation strategies such as cosolvent systems, emulsification, micellization, liposome formation, and inclusion in cyclodextrins have been studied. Biodegradable nanoparticle formulations using poly(lactic-co-glycolic) acid have been studied by Wang et al. 1996 [25] and [26] and have shown comparable activity to traditional formulations and much faster administration. Paclitaxel could be incorporated at very high loading efficiencies, nearing 100%, using the nanoprecipitation method using acetone and PLGA [27]. These same nanoparticles, at 117–160 nm diameter, released over half of their drug in vitro in the first 24 h of release with a much slower release rate over the next 4 days. Cellular studies showed up to a 70% loss of viability in NCI-H69 human small cell lung cancer cells at levels as low as 0.025 μg/ml. Another group has added vitamin E TGPS (d-α-tocopheryl polyethylene glycol 1000 succinate) as an emulsifier and matrix component to PLGA nanoparticles for paclitaxel release [28]. These particles ranged from 369 to 1764 nm in diameter and from 43% to 84% encapsulation efficiency, depending on the particular formulation prepared. The in vitro release from these nanoparticles was followed for 1 month and typically the burst of release seen was 15% in the first day, following by a very constant rate of release for the duration of the study, to where usually 60% of the paclitaxel had released by 1 month.

3.2. Doxorubicin

One of the most potent and widely used anticancer drugs is doxorubicin which works by inhibiting the synthesis of nucleic acids within cancer cells [29]. Doxorubicin has a number of undesirable side effects such as cardiotoxicity and myelosuppression which leads to a very narrow therapeutic index. Various researchers have studied ways to target doxorubicin delivery to cancer tissues or at least to diminish its side effects. Conjugates of dextran and doxorubicin have been encapsulated in chitosan nanoparticles of ~ 100 nm diameter. It was found that mice injected intravenously with both dextran-doxorubicin conjugates and the conjugates encapsulated in chitosan nanoparticles showed a decrease in the tumor volume after 4 weekly injections with the tumor volume of the mice treated with the encapsulated conjugate being only 60% of that of the tumors treated with the conjugate alone. Treatment with doxorubicin alone did not decrease the tumor volume [30]. Another method being studied for cancer treatment is neutron-capture therapy using gadolinium [31]. In vitro cellular studies of chitosan-encapsulated gadopentetic acid in nanoparticles has shown that these nanoparticles will be taken up by L929 fibroblast cells, B16F10 melanoma cells and SCC-VII squamous cell carcinoma cells through endocytosis at levels that are orders of magnitude higher that a dimeglumani gadopentate aqueous solution (Magnevist®, the currently available formulation of gadolinium).

One group has conjugated doxorubicin to PLGA and formed nanoparticles from these conjugates [29]. Nanoparticles were prepared of 200–250 nm diameter with in vitro release up to 1 month. Analysis in vivo of injected nanoparticles as compared with daily doxorubicin injections showed that a single injection of doxorubicin-PLGA conjugate nanoparticles could suppress tumor growth for up to 12 days, although not quite as well as daily doxorubicin injections at the levels tested.

3.3. 5-Fluorouracil

Incorporation of 5-fluorouracil has also been achieved using dendrimers of poly(amidoamine) modified with mPEG-500. The hydrophilicity of

the 5FU allowed it to complex with the dendrimers after simply incubating the polymer with the drug. For in vitro studies, PEGylated formulations showed release over 144 h (6 days) while non-PEGylated formulations had completed their release within 1 day. Studies in rats of intravenously administered formulations showed that free 5-FU was cleared from the bloodstream within 1.75 h. Those given the dendrimer formulations, however, showed 5-FU clearance only after 7 h for non-PEGylated systems and 13 h for PEGylated systems, both at fairly constant levels for the duration of the release. This confirms the formulations ability to control the 5-FU release in vivo and the extension of that release by PEGylation of the polymers in the formulation [32].

3.4. Antineoplastic agents

Camptothecin-based drugs, specifically irinotecan (Camptosar) and topotecan (Hycamptin) have been approved by the FDA and are used most often either in conjunction with 5-fluorouracil as a first therapy or sometimes used alone after 5-fluorouracil has failed. Analogs of these molecules have shown up to 1000-fold higher activity but are a great challenge to delivery because of their extreme hydrophobicity [33]. Nanoparticles of 100–375 nm diameter were prepared with the SN-38 analog of irinotecan in lipid-based nanoparticles. The weight of tumors was followed for mice injected twice weekly for 2 weeks (4 doses) or daily for 10 days (10 doses) with Camptosar as compared with encapsulated SN-38 twice weekly for 2 weeks (4 doses) at two different particle sizes. The longest tumor regression and survival was seen for mice injected with nanoparticles ∼ 375 nm in diameter (65 days survival, 1.98 mg SN-38/mouse), followed by those injected daily with Camptosar (51 days survival, 9 mg irinotecan/mouse) and those injected with nanoparticles ∼ 100 nm diameter (48 days survival, 1.51 mg SN-38/mouse). The control mice survival was 22 days.

3.5. Gene delivery

Other ligands that have shown selective targeting to cancer cells are transferrin (Tf) and epidermal growth factor (EGF) [34–36]. Complexes for DNA

delivery composed of polyethylenimine (PEI) linked to poly(ethylene glycol) (PEG) which are then coated with either Tf or EGF were prepared with nanoparticle diameters ranging from 49 to 1200 nm diameter. Plasmid pCMVLuc which codes for luciferase production was incorporated into these nanoparticles and in vivo studies in mice showed that the gene expression from administration of targeted systems was 10–100 higher in tumors than in other organs [37].

4. Targeting to specific organs or tumor types

One of the greatest challenges is defining the optimal targeting agent or agents to selectively and successfully transport nanoparticle systems to cancerous tissue. These strategies also then rely on the targeting agents' or ligands' capability to bind to the tumor cell surface in an appropriate manner to trigger receptor endocytosis. The therapeutic agents will thereby be delivered to the interior of the cancer cell.

4.1. Breast cancer

An example of the type of work which can be done to identify the ideal ligands for targeting is the development of a strategy to select internalizing antibodies from phage libraries [38]. This technique was used to identify two antibodies (F5 and C1) to the breast tumor cell line SK-BR-3 that bind to ErbB2, a growth factor that is overexpressed in 20–30% of human breast carcinomas and also in other adenocarcinomas [39]. A research study used Doxil (commercial liposomal doxorubicin formulation from Alza) with which a modified PEG conjugated to antibody F5 had been incubated to form a coupled liposome system. Comparison in vivo in mice treated with Doxil or F5-coupled Doxil showed a faster and greater regression in tumor volume for F5-containing Doxil over unmodified Doxil [39].

4.2. Liver

A promising receptor for liver targeting is the asialoglycoprotein receptor (ASGP-R, galactose re-

ceptor). Work by Kim et al. [40] describes nano-particles that use the galactose moiety from lacto-bionic acid, biotin and diamine-terminated poly (ethylene glycol) which exhibit in vitro release of A11-trans-retinoic acid (a model cancer drug) at a fairly constant rate over 1 month.

4.3. Folate receptors

The cell surface receptor for folic acid (folate receptor) is inaccessible from the circulation to healthy cells but is expressed on the surface of cancer cells making it a possible target for a number of types of cancer [41]. These therapies include targeting of immunotherapies using folic acid-derived antibodies or Fab/scFv fragments to the T cell receptor. Some researchers have also studied preparing cancer vaccines to treat folate-receptor positive tumors by developing a vaccine against the folate receptor. This approach in treating folate receptor-positive lung metastases in mice has produced cures in up to 56% of tumor-bearing mice [42].

5. Imaging for cancer

Many of the same techniques used to target delivery of drugs to cancerous tissues may also be used to target imaging agents. In fact, as targeted delivery systems approach the stage where they can be used clinically, primary assessment of the utility of a particular formulation in a particular patient may be made with imaging agents to verify that the delivery system goes primarily to the cancerous tissues before any drug regimen is begun.

Studies using vasoactive intestinal peptide (VIP), whose receptors are five times more numerous in breast cancer cells than normal breast cells, as a targeting agent for sterically stabilized liposomes have shown that both passive and active targeting to breast cancer cells will occur in vivo in rats [43]. Specifically, for liposomes of $\sim$ 110 nm diameter with or without 10 μg/μmol VIP, the amount of the encapsulated radionucleotide Tc99 m-HMPAO the uptake of non-targeted liposomes in breast cancer tissue was three times that of normal breast tissue and targeted liposomes accumulated at a rate of

about six times that in normal breast tissue, all measured 27 h post-injection.

6. Conclusions

Research activity aimed towards achieving specific and targeted delivery of anti-cancer agents has expanded tremendously in the last 5 years or so with new avenues of directing drugs to tumors as well as new types of drugs. The first of these creative treatment methods have made it to the clinic and hopefully are well on their way to improving the length and quality of life for cancer patients. However, there is a great deal more that can be done to treat and perhaps prevent advanced cancer by treating it in as early a stage as possible. This will require superior detection and targeting methods which many of the researchers mentioned here will undoubtedly pursue and hopefully achieve.

Appendix A. Endogenous angiogenesis stimulators

Acidic fibroblast growth factor (FGF-1)	Potent general mitogen and motogen for endothelial and tumor cells
Basic fibroblast growth factor (FGF-2)	One of the most potent mitogens and motogens for endothelial and tumor cells
Epidermal growth factor	Stimulates proliferation and increases motility of endothelial cells
Hepatocyte growth factor	Secreted by mesenchymal-derived cells, a mitogen and motogen for endothelial cells and inducer of VEGF
Platelet-derived endothelial cell growth factor (thymidine phosphorylase)	Promotes endothelial cell migration and angiogenesis
Platelet-derived growth factor	Stimulates proliferation and motility of endothelial cells
Transforming growth factor α	Mitogen for endothelial cells, stimulates angiogenesis
Transforming growth factor β	Stimulates production and activation of MMP-2, but also shown to inhibit endothelial proliferation in vivo
Vascular endothelial growth factor	Mitogen, motogen and survival factor for endothelial cells

Appendix A (*continued*)

Angiogenin	Angiogenic factor secreted by tumor cells
Angiopoietin-1	Secreted pro-angiogenic factor that binds VEGF receptor (Tie-2) and stimulated vascular maturation
Interleukin-6	Pro-angiogenic factor expressed transiently by capillary network around follicles
Interleukin-8	Mitogen for endothelial cells secreted by cancer cells
Matrix metalloproteinase-2 (MMP-2)	Secreted by endothelial and tumor cells for wound healing, tumor invasion and tissue remodeling
Matrix metalloproteinase-9 (MMP-9)	Secreted by endothelial and tumor cells for wound healing, tumor invasion and tissue remodeling
Tumor necrosis factor α	Pro-inflammatory cytokine shown to stimulate angiogenesis

Appendix B. Endogenous inhibitors of angiogenesis

Angiopioetin-2	May be involved in maturation of neovessels, blocks effect of angiopoietin-1
Interferon-$\alpha-\beta$	Block production of pro-angiogenic molecules
Interferon-γ	Induced by IL-12, blocks induction of angiogenesis by FGF, induces production of IP-10
Interferon-γ-inducible protein (IP-10)	A CXC chemokine induced by interferon-γ, active anti-angiogenic moiety
Interleukin 10	Stimulates TIMP-1 expression, inhibits secretion of MMP-2
Interleukin 12	Inhibits angiogenesis in vivo by stimulating interferon-γ to induce IP-10, activates natural killer cell cytotoxicity of endothelial cells
Platelet factor-4	Member of CXC chemokine family that inhibits endothelial cell proliferation and migration
Soluble flt-1	Soluble form of VEGF receptor capable of binding VEGF molecules in the circulation and reducing net function
Thrombospondin-1	Endogenous inhibitor of angiogenesis down regulated with p53 mutation
Tissue inhibitor of matrix metalloproteinase-1, -2 (TIMP-1,-2)	Able to block activity of some MMPs, decrease induction of endothelial cell migration and invasion

Appendix B (*continued*)

Angiostatin	Plasminogen fragment containing kringle domains that inhibit angiogenesis
Endostatin	Fragment of type XVIII collagen that inhibits endothelial cell proliferation and angiogenesis
Prolactin fragment	Inhibits endothelial cell proliferation
Secreted protein, acidic and rich in cysteine (SPARC)	Inhibits cell attachment and spreading, fragments shown to have anti-angiogenic activity

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