

Strategies of Targeting Tumors and Cancers

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Abstract: Targeted cancer therapies use drugs that specially reach at the affected site block the growth and spread of cancer. They interfere with specific molecules involved in carcinogenesis (the process by which normal cells become cancer cells) and tumor growth. By focusing on molecular and cellular changes that are specific to cancer, targeted cancer therapies may be more effective than current treatments and less harmful to normal cells. Targeted cancer therapies interfere with cancer cell growth and division in different ways and at various points during the development, growth, and spread of cancer.

The present article provides an overview of various aspects of cancers and tumors that include causes of the diseases and their underlying biology, existing methods of treatment, major strategies of cancer and tumor targeting and mechanisms of their mode of actions. The review article also presents a current state-of –the art of the cancer targeting approaches and discusses various types of advanced targeting techniques like pH, temperature and magnetic targeting. A brief account of recent literature pertaining to cancer targeting is also discussed.

Keywords: Cancer, Targeted Therapy, Tumors, Drug Targeting, Chemotherapy.

1. INTRODUCTION

Statistical survey indicates that more than 11 million people are diagnosed with cancer each year, and cancer accounts for about 7 million deaths/year (12.5% of deaths worldwide), making this disease a huge factor in worldwide mortality. The incidence of cancer is expected to increase continuously as the world population ages, and it has been estimated that there will be 16 million new cancer cases every year by 2020 [1]; and despite tremendous efforts to treat cancer, there has been very little actual improvement in cancer therapeutics over the past 50 years.

The first description of cancer is found in an Egyptian papyrus and dates back to approximately 1600 BC. It was regarded as an incurable disease until the nineteenth century, when surgical removal was made more efficient by anesthesia, improved biomedical techniques and histological control. Before 1950, surgery was most preferred means of treatment, however, after 1960, radiation therapy started being used to control local disease. However, over the time it was realized that neither surgery nor radiation or the two in combination could adequately control the metastatic cancer and that, for treatment to be effective, therapy needed to reach every organ of the body. Therefore, current efforts to cure cancer have been focusing on drugs, biological molecules and immune mediated therapies. The introduction of nitrogen mustard in the 1940s can be considered as

the origin of antineoplastic chemotherapy targeting all tumor cells [2]. Today, more than 30 years later, although improved mortality rate or prolonged survival time for metastatic cancer have not been achieved as expected, however, the characteristics and pathways of different tumor entities have been identified. This knowledge is now used to generate specific tumor therapies either by directly targeting the proteins involved in the neoplastic process or by targeting drugs to the tumor.

Recent advancements in the knowledge of molecular biology of cancer and pathways involved in malignant transformation of cells are revolutionizing the approach to cancer treatment with a focus on targeted cancer therapy. The newer approaches to cancer treatment not only supplement the conventional chemotherapy and radiotherapy but also aim to prevent damage to the normal tissues and overcome drug resistance. Innovative methods of cancer treatment require new concepts of drug delivery in cancer. The concept of drug targeting is used to improve the therapeutic index of drugs by increasing their localization to specific organs, tissues or cells and by decreasing their potential toxic side effects at normal sensitive sites [3]. As in the field of cancer therapy, chemotherapeutic agents have toxic side effects for tumor cells as well as for normal cells; the targeted delivery of these agents to diseased sites would enable the use of higher doses for increasing therapeutic efficacy [4]. Controlled drug delivery involves the association of a drug with a carrier system, thereby allowing modulation of the pharmacokinetic properties and biodistribution of the drug [5]. Targeted drug

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delivery systems can control the site of action but are usually unable to dictate the release kinetics of the drugs in a predictable manner. Controlled release systems generally can control the rate of drug release but cannot control the fate of the drug once it has been released. Targeted therapies are designed to interfere with specific aberrant biological pathways involved in tumorigenesis. This is in contrast with the generalized cytotoxic effects of standard chemotherapy. Various mechanisms relevant in carcinogenesis are exploited by targeted therapies, such as angiogenesis, cell growth signaling and apoptosis.

In order to substantially improve effective cancer therapy, one must vastly improve knowledge of cancer pathophysiology, discover new anticancer drugs, and develop novel biomedical technologies. Consequently, cancer therapy has become a multidisciplinary challenge requiring close collaboration among clinicians, biological and material scientists, and biomedical engineers. This article focuses on the target therapy found to be significantly efficacious and the novel strategies with clinical promise.

2. DEFINITION AND TYPES OF CANCER

Molecular and cellular analyses have demonstrated unregulated growth, maturation, and cell survival as basic characteristics of many types of cancers. Cancer, an abnormal growth of cells anywhere in the body, is often described as a group of more than 100 different diseases that have similar characteristics. Cancer induces when healthy cells begin to change and grow uncontrollably, forming a mass called a tumor as shown in Figure 1. Some cancers, however, such as

blood cancers, do not form tumors. The change from a normal cell to a cancerous one is largely the result of specific mutations to genes that control cell division or delay the normal processes by which cells die. Tumors are broadly classified into two types benign tumors and malignant tumors (Figure 2). Benign tumors are not “cancer” and do not spread widely throughout the body, although they can grow substantially and damage surrounding tissues, if not treated [6]. Malignant tumor is a group of cells displaying *uncontrolled growth* (division beyond the normal limits), *invasion* (intrusion on and destruction of adjacent tissues), and sometimes *metastasis* (spread to other locations in the body *via* lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize. Only malignant tumor cells and infections have the established capacity to metastasize [7].

3. CAUSE OF CANCER

The vast majority of cancers are sporadic. There is no clear cause why one person gets cancer and another does not. Cancer is a genetic disease [8]. Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells [9]. The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome. Although environmental and other non-genetic factors have roles in many stages of tumorigenesis, it is widely accepted that cancer arises because of mutations in cancer-susceptibility genes. These genes belong to one of three classes [8,10]: gatekeepers, caretakers and landscapers.

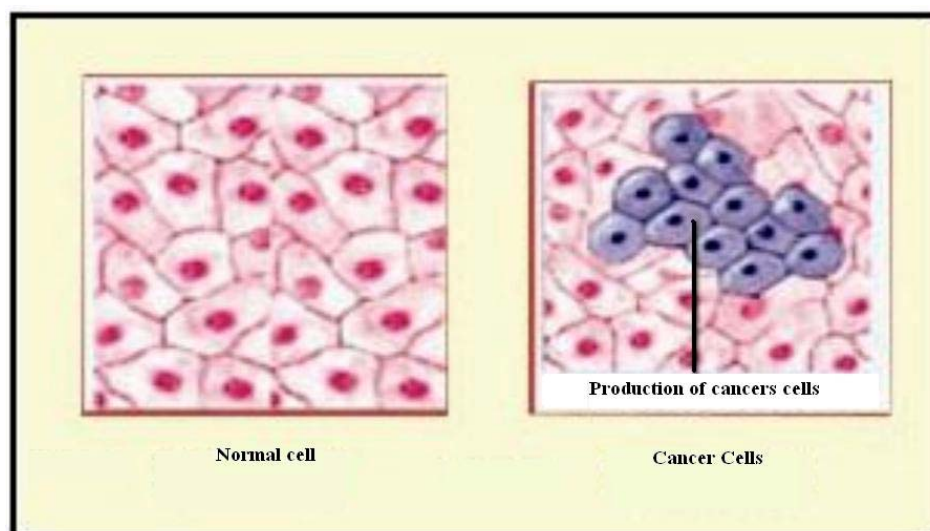


Figure 1: Difference between normal and cancer Cells.

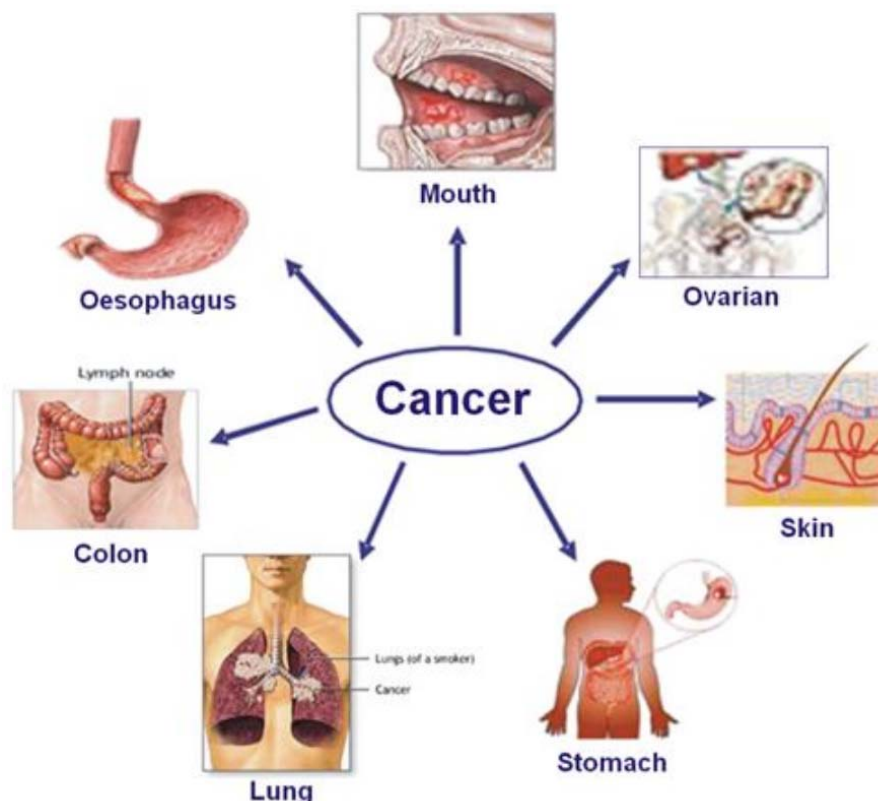


Figure 2: Different type of cancers.

Gatekeepers directly regulate growth and differentiation pathways of the cell and comprise oncogenes and tumour-suppressor genes (TSGs). Proto-oncogenes are normal genes that are involved in cell growth and division. Changes, or mutations in these genes lead to the development of oncogenes. Cancer-promoting oncogenes are typically activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the ability to become established in diverse tissue environments [6,11,12].

Caretakers, by contrast, promote tumorigenesis indirectly [13, 14]. They function in maintaining the genomic integrity of the cell. Mutation of caretakers can lead to genetic instability and the cell rapidly accumulates changes in other genes that directly control cell birth and death [15].

Landscaper defects do not directly affect cellular growth, but generate an abnormal stromal environment that contributes to the neoplastic transformation of cells [8,16]. An abnormal stroma can be regarded as a classical promoter in the terminology of carcinogenesis, in that the dysfunction of normal epithelial–mesenchymal interactions increases the probability that the preneoplastic lesion will progress to malignancy.

On the other hand, in the terminology of development, the environment provided by the abnormal stroma may be considered ‘permissive’ for tumorigenesis by leading to the selection of cells with altered survival characteristics [17].

The interaction between stromal cells and tumor cells is known to play a major role in cancer growth and progression. Stromal cells near the bottom of the epidermis (the very top layer of the skin) release growth factors that promote cell division. Cell growth and differentiation Growth factor (GF) refers to a group of proteins that facilitate cell proliferation and growth by adhering to specific growth factor receptors on the surface of that cell, such as receptor tyrosine kinase (RTK). Epidermal growth factor (EGF) promotes cell proliferation in the epithelial layer of the skin which primarily comprises keratinocytes at different stages of differentiation. EGF receptor works in concert with ErbB proto-oncoprotein, which delivers growth-stimulating signals for cell proliferation. Many varieties of human cancers indicate hyperactive signaling of GF receptors [6, 18]. For example, when growth factors (GFs) attach themselves to corresponding growth factor receptors (GFRs) on the surface of the cell, a process carried out by proteins signals the cell to divide. Damaged proteins may not respond to these

normal signals, over-respond to normal signals, or otherwise fail to carry out their normal functions. When these failures or errors occur, cancer develops causing it to reproduce excessively and allowing that cell to live longer than normal cells. As cancer cells continue to reproduce, their daughter cells also contain the cancer as well.

The alteration of one gene, however, does not suffice to give rise to full-blown cancer. For progression towards malignancy and invasion, further mutational hits are necessary [19–21]. So, the risk of cancer development depends not only on mutations initiating tumorigenesis, but also on subsequent mutations driving tumour progression. These mutations occur due to a complex mix of factors related to lifestyle, heredity and environment.

4. MAIN FEATURES OF CANCER BIOLOGY

Normal cells perfectly fit their environment and respond to external signals *via* tightly regulated pathways that either trigger or repress growth. Actually, tumorigenesis appears as a multistep mechanism that reflects the genetic alterations driving progressively a normal tissue to malignancy. The genetic diversity usually presented by cancer cells does not correlate with the clinical observations where a common invasive behavior including uncontrolled growth and destruction of normal tissues are noted. Such a paradox can be explained by the selective barriers existing within a tumour (hypoxia, malnutrition, hormonal fluctuations, attacks of the immune system, etc) that lead to the selection of adapted cells [22]. Interestingly, this evolutionary process seems to be related to the manifestation of six main alterations in cell physiology, which together define the progression of most human malignancies as nicely described by Hanahan and Weinberg [23].

- I. **Self sufficiency in growth signals:** Normal cell proliferation depends upon the presence of growth factors produced outside of the cell. However, one of the key characteristics of the tumor cell is its capacity for proliferation without dependence on external growth factors. Tumor cells may proliferate by either internal production of growth factors or by responding to levels of external growth factors not usually sufficient to produce proliferation in normal cells.
- II. **Insensitivity to antigrowth signals:** In normal tissue, the stability of the cell population is maintained by a host of signals and factors

inhibiting cell proliferation and differentiation. For cancer cells to survive and replicate, these antigrowth signals must be avoided.

- III. **Tissue invasion and metastasis:** The third hallmark of cancer cells is tissue invasion and metastasis (spreading) which is a major source of mortality in cancer patients. Cancer spreads when gene mutations allow transformed cells to break free from the primary tumor and travel through the bloodstream to establish a new colony of cells at another location.
- IV. **Limitless replicative potential:** Many, if not all, normal human cells are programmed to limit their own replication. However, for cells to form a potentially life-threatening tumor, the mechanisms that normally limit replication must be disrupted. For a tumor cell population to expand, it must develop unlimited replicative potential, effectively gaining "immortality."
- V. **Sustained angiogenesis:** In normal tissue, continued cell function is dependent on the availability of oxygen and nutrients and the removal of metabolic waste through the capillary beds. Angiogenesis (the process by which new blood vessels are formed) is not an inherent property of most cells in small, localized neoplasms. To develop into larger, potentially metastatic tumors, angiogenic ability must be acquired.
- VI. **Evading apoptosis:** In normal tissue, the stability of the cell population is maintained through a process of programmed cell death, or apoptosis, which is latent in virtually all cell types throughout the body. Acquiring resistance to apoptosis is one of the key mechanisms by which cancer cells maintain proliferation and is thought to be a critical survival factor for the majority of tumors.

Supporting this hypothesis, it has been recently shown that a simple network of well-defined genetic events is sufficient to convert a healthy cell into a tumorigenic state [24]. The main physiological changes presented above represent novel capabilities and are acquired during tumour development through multiple mutations.

5. THE EARLY STAGE DETECTION OF CANCER

It is found that more than 60% of cancer patients, cancer is not treated and diagnosed until cancer cells

have already invaded surrounding tissues and metastasized throughout the body. The ability of physicians to effectively treat and cure cancer is directly dependent on their ability to detect cancers at earliest stages. The early detection is crucial for its ultimate control and prevention. The current and future treatment strategies will have a higher probability of curing of various cancer depends upon early stage detection. There are following techniques which are used as early stage detection.

5.1. Biomarkers

Gene mutations, alterations in gene translation and gene transcription serve as biomarkers for disease. Biomarkers are important techniques for detection and monitoring of cancer because they act as hallmarks for physiological status of cell at a given time and changed during the disease process [25]. Cancer-associated autoantibodies represent appealing biomarkers for early detection of cancer. Autoantibodies may develop early in carcinogenesis when tumor-associated antigens appear on premalignant or malignant lesions. Antibody responses can produce relatively high concentrations in circulation with a long circulation time, and they can be detected with sensitive and specific methods. In contrast, antigens produced by small premalignant or malignant lesions are generally produced in vanishingly small levels, which, due to dilution and clearance from blood, may not be detectable. Cancer-associated autoantibodies characterized to date have been found to bind intracellular proteins with functions important in cell cycle regulation, such as GPR78, p53, NY-ESO-1, and CDC25, but also some cell membrane glycoproteins such as MUC1, HER2, and mesothelin [26].

5.2. MRI Imaging

Early detection of adenomatous colonic polyps is a major concern in the prevention of colon cancer. Colorectal cancer can largely be prevented by the early detection and removal of adenomatous polyps. A variety of colorectal cancer screening modalities are available, including stool-based tests and radiological and endoscopic examinations of the colon. These methods are considered to be either lacking in sensitivity or invasive, and colon cancer continues to be a major cause of death in the western world. Novel fluorescent nanoparticles with potential to improve neoplasm detection sensitivity may prove to be a valuable tool in early detection of colon tumors [27].

Magnetic resonance imaging (MRI) is a noninvasive technique which is used for showing inside images of

an object, basically depends on the relaxation properties of protein nuclei in water and lipids. It has some advantages such as (i) to employ nonionizing radio frequency signals to obtain its images and is best matched to noncalcified tissues in the body, (ii) to be able to detect various features in tissues *via* varying scanning parameters, (iii) to create cross sectional images in any images besides oblique planes, (iv) to be superior to detect and identify tumors [28].

Magnetic resonance imaging (MRI) contrast agents have made a significant impact in the use of MRI for various clinical indications. Superparamagnetic iron oxide (SPIO) composed of nano sized iron oxide crystal coated with biopolymers like dextrin or carboxydextran affects the MRI signal properties of surrounding tissues, therefore, used as MRI contrast agents. There are two clinically approved SPIO agents are ferumoxids (feridex in the USA, Endorem in Europe) with a particle size of 120 to 180 nm, and ferucarbotran (Resovist) with a particle size of about 60nm [29].

Zhang *et al.* [30] synthesized superparamagnetic nanoparticles by iron oxide particles coated by gum Arabic for evaluating their magnetic resonance imaging as well as drug delivery abilities. They found that gum Arabic coated magnetic nanoparticles (GA-MNP) possessed a superior stability than commercial magnetic nanoparticles (MNP) products currently used in MRI upon storage in aqueous media. A preliminary *in vivo* testing result show that GA-MNP act as MRI visible drug carrier which achieved both magnetic tumor targeting and intracellular drug delivery under the application of external magnetic field.

6. TYPES OF TREATMENT

Cancer refers to a class of diseases; therefore there is not a single cure for cancer like other infectious diseases. Cancer [31] can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy or other methods. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the patient. Complete removal of the cancer without damage to the rest of the body is the goal of treatment.

6.1. Chemotherapy

Chemotherapy is the treatment of cancer with drugs ("anticancer drugs") that can destroy cancer cells. In current usage, the term "chemotherapy" usually refers

to cytotoxic drugs which affect rapidly dividing cells. This means that it also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract and hair follicles; this results in the most common side effects of chemotherapy—myelosuppression (decreased production of blood cells), mucositis (inflammation of the lining of the digestive tract) and alopecia (hair loss). Unlike surgery, chemotherapy affects the entire body, not just a specific part. It works by targeting rapidly multiplying cancer cells. Unfortunately, other types of cells in our bodies also multiply at high rates, like hair follicle cells and the cells that line our stomachs. This is why chemotherapy can cause side effects like hair loss and an upset stomach. Chemotherapy is most commonly given by pill or intravenously (IV), but can be given in other ways. A single type of chemotherapy, or a combination of drugs, may be prescribed for a specific length of time. Like surgery, chemotherapy can be prescribed alone, in conjunction with radiation therapy or biologic therapy. Chemotherapy can:

- Make a tumor smaller before surgery or radiation therapy. This is called **neo-adjuvant chemotherapy**.
- Destroy cancer cells that may remain after surgery or radiation therapy. This is called **adjuvant chemotherapy**.
- Help radiation therapy and biological therapy work better.
- Destroy cancer cells that have come back (**recurrent** cancer) or spread to other parts of body (**metastatic** cancer).

Drugs used in cancer chemotherapy all have narrow therapeutic indices. This means that the dose levels at which these drugs significantly affect a tumour are close to those levels at which unacceptable toxic side-effects occur. Therefore, more effective treatments result from balancing the beneficial and adverse effects of a combination of different drugs, administered at various dosages over a treatment period [32]. This is called "combination chemotherapy"; most chemotherapy regimens are given in a combination [31]. The treatment of some leukaemias and lymphomas requires the use of high-dose chemotherapy, and total body irradiation (TBI). This treatment ablates the bone marrow, and hence the body's ability to recover and repopulate the blood. For this reason, bone marrow, or peripheral blood stem cell

harvesting is carried out before the ablative part of the therapy, to enable "rescue" after the treatment has been given. This is known as autologous stem cell transplantation. Alternatively, hematopoietic stem cells may be transplanted from a matched unrelated donor (MUD).

The beneficial effects of cancer chemotherapy correspond to treatment objectives which oncologists want to achieve by means of administering anticancer drugs. A cancer chemotherapy treatment may be either curative or palliative. Curative treatments attempt to eradicate the tumour; palliative treatments, on the other hand, are applied only when a tumour is deemed to be incurable with the objective to maintain a reasonable quality of life for as long as possible. The adverse effects of cancer chemotherapy stem from the systemic nature of this treatment: drugs are delivered *via* the bloodstream and therefore affect all body tissues. Since most anti-cancer drugs are highly toxic, they inevitably cause damage to sensitive tissues elsewhere in the body. In order to limit this damage, toxicity constraints need to be placed on the amount of drug applied at any time interval, on the cumulative drug dosage over the treatment period, and on the damage caused to various sensitive tissues [33]. In addition to toxicity constraints, the tumour size (i.e. the number of cancerous cells) must be maintained below a lethal level during the whole treatment period for obvious reasons. The goal of cancer chemotherapy therefore is to achieve the beneficial effects of treatment objectives without violating any of the above mentioned constraints.

There are many different types of drugs that qualify as chemotherapy agents, and the drug that is chosen is dependant on what type of cancer will be treated. The drugs are classified into five major categories based on the way the drugs affect cell chemistry, and which stage of the cells life cycle the drugs effect. The categories are alkylating agents, antimetabolites, anthracyclines antibiotics, plant (vinca) alkaloids, topoisomerase inhibitors, and other antitumour agents [31]. Some examples of drugs are cisplatin, doxorubicin, paclitaxel, 5-fluorouracil, adriamycin, irinotecan, etoposide, chlorambucil etc. All of these drugs affect cell division or DNA synthesis and function. Depending on the drug chosen, chemotherapy will affect malignant cells in one of three ways:

- Damage the DNA of the cancer cells so they can no longer reproduce. This is done by altering the

DNA structure in the nucleus of the cell preventing replication.

- During the S phase of cell life, inhibit the synthesis of new DNA strands so that no cell replication is possible. This occurs when the drugs block the formation of nucleotides that are necessary for new DNA to be created.
- Stop the mitotic processes of the cell so that the cancer cell cannot divide into two cells. The formation of mitotic spindles is necessary to move the original DNA and the replicated DNA to opposite sides of the cell so the cell can divide into two cells.

In general, the clinical application of conventional anticancer drugs involves high patient risks because the drugs are not specific to cancer cells. Most patients must tolerate severe side effects, decreased quality of life, and repeated treatments. The inefficiency and side effects of chemotherapy have been primarily associated with the formulation and biodistribution of the drug, toxicity to normal cells, and the acquisition of drug resistance by the cancer cells. Thus, researchers are continuously seeking to overcome these issues.

6.2. Surgery

In theory, non-hematological cancers can be cured if entirely removed by surgery, but this is not always possible. When the cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible. In the Halstedian model of cancer progression, tumors grow locally, and then spread to the lymph nodes, then to the rest of the body. This has given rise to the popularity of local-only treatments such as surgery for small cancers. Even small localized tumors are increasingly recognized as possessing metastatic potential.

Examples of surgical procedures for cancer include gastrectomy [34] for gastric cancer, mastectomy [35] for breast cancer and prostatectomy for prostate cancer. The goal of the surgery can be either the removal of only the tumor, or the entire organ. A single cancer cell is invisible to the naked eye but can regrow into a new tumor, a process called recurrence. For this reason, the pathologist will examine the surgical specimen to determine if a margin of healthy tissue is present, thus decreasing the chance that microscopic cancer cells are left in the patient. In addition to removal of the primary tumor, surgery is often

necessary for staging, e.g. determining the extent of the disease and whether it has metastasized to regional lymph nodes.

6.3. Radiation Therapy

Radiation therapy (also called radiotherapy, X-ray therapy, or irradiation) is the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy [36] can be administered externally *via* external beam radiotherapy (EBRT) or internally *via* brachytherapy. The effects of radiation therapy are localised and confined to the region being treated. Radiation therapy injures or destroys cells in the area being treated (the "target tissue") by damaging their genetic material, making it impossible for these cells to continue to grow and divide. Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly. The goal of radiation therapy is to damage as many cancer cells as possible, while limiting harm to nearby healthy tissue. Hence, it is given in many fractions, allowing healthy tissue to recover between fractions.

Radiation therapy may be used to treat almost every type of solid tumor, including cancers of the brain, breast, cervix, larynx, lung, pancreas, prostate, skin, stomach, uterus, or soft tissue sarcomas. Radiation is also used to treat leukemia and lymphoma. Radiation dose to each site depends on a number of factors, including the radiosensitivity of each cancer type and whether there are tissues and organs nearby that may be damaged by radiation. Thus, as with every form of treatment, radiation therapy is not without its side effects.

6.4. Hormonal Therapy

The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial.

6.5. Immunotherapy

Cancer immunotherapy refers to a diverse set of therapeutic strategies designed to induce the patient's own immune system to fight the tumor. Contemporary methods for generating an immune response against tumours include intravesical BCG immunotherapy for

superficial bladder cancer, and use of interferons and other cytokines to induce an immune response in renal cell carcinoma and melanoma patients. Vaccines to generate specific immune responses are the subject of intensive research for a number of tumours, notably malignant melanoma and renal cell carcinoma. Sipuleucel-T is a vaccine-like strategy in late clinical trials for prostate cancer in which dendritic cells from the patient are loaded with prostatic acid phosphatase peptides to induce a specific immune response against prostate-derived cells.

Allogeneic hematopoietic stem cell transplantation ("bone marrow transplantation" from a genetically non-identical donor) can be considered a form of immunotherapy, since the donor's immune cells will often attack the tumor in a phenomenon known as graft-versus-tumor effect. For this reason, allogeneic HSCT leads to a higher cure rate than autologous transplantation for several cancer types, although the side effects are also more severe.

6.6. Photodynamic Therapy

Photodynamic therapy (PDT) relies on the activation of a photosensitizing agent with visible or near-infrared (NIR) light. Upon excitation, a highly energetic state is formed which upon reaction with oxygen affords a highly reactive singlet oxygen capable of inducing necrosis and apoptosis in tumor cells [37]. The tumor selectivity of porphyrin photosensitizers has been attributed to its characteristic leaky vasculature, compromised lymphatic drainage, and high degrees of newly synthesized collagen and lipid content, both for which porphyrins have an affinity for. PDT has been shown to reduce tumors by direct cell killing, destruction of tumor neovasculature, and triggering of an acute inflammatory response that attracts leukocytes to the tumor [38].

6.7. Photothermal Therapy

With the advent of metal nano-particles during the 1990's, photothermal ablation has burgeoned into a new niche of minimally invasive tumor therapies [39]. Gold-based nanoparticles have been developed that strongly absorb light in the near-infrared region, facilitating deep optical penetration into tissues, generating a localized lethal dose of heat at the site of a tumor [40]. The first methods for preparing metal-encapsulated dendrimers for use in biomedical applications were reported within the last decade with the goal of adding a finer degree of control for tuning

the biological interactions elicited by the metal particles, including improved biocompatibility, retention, and ease of surface modification for potential use as biomarkers, contrast agents, and for photothermal therapy [41].

6.8. Targeted or Biologic Drug Delivery

Targeted therapies represent a new type of cancer treatment, in which specific molecules in cancer cells are blocked to slow or stop the growth of cancer. Biologic therapy is a term for drugs that target characteristics of cancerous tumors. Some types of targeted therapies work by blocking the biological processes of tumors that allow tumors to thrive and grow. Other types of therapies cut off the blood supply to the tumor, causing it to basically starve and die because of a lack of blood. It is given in conjunction with other cancer treatment. Compared with conventional chemotherapy, these drugs are more specific and cause fewer side effects commonly associated with chemotherapy, such as infections, weakness, and changes to blood counts. Drug targeting is the delivery of a drug to a specific site in the body where desirable effects can be achieved without exposing other sites to possible drug toxicity. This is an important distinction from the basic targeting concept, where the specific drug receptor is the target and the objective is to improve fit, affinity, and binding to the specific receptor that will ultimately trigger the pharmacological activity. This distinction is made since the overall distribution of many drug receptors does not follow the various diseases. Actually, most of the time, drug toxicity is receptor related and receptor mediated; thus, improving intrinsic drug affinity and activity, as well as receptor binding, does not improve the therapeutic index.

In principle, drug targeting can be achieved by physical, biological, or molecular systems that result in high concentrations of the pharmacologically active agent at the patho-physiologically relevant site. If successful, the result of the targeting would be a significant reduction in drug toxicity, reduction of the drug dose, and increased treatment efficacy. All in all, it is evident that with a biologically active agent of reasonable activity at hand, targeting to the site of action should be superior to molecular manipulations aimed at refining the receptor substrate interactions. In the field of oncology, the targeted delivery of chemotherapeutics to tumor cells translates to significantly reduced side effects compared to systemic delivery where healthy tissue such as the liver, spleen,

kidneys, and bone marrow can accumulate toxic levels of drug. Biological drugs and targeted therapies are aimed at a specific cellular target, such as small molecules that inhibit a specific protein molecule that is a key player in signal transduction, in apoptosis, in the cell cycle or in other important cellular pathways. Different types of targeted therapies are shown in Figure 3.

6.8.1 Major Schemes of Targeted Drug Delivery

Following strategies may be adopted to achieve targeted drug delivery:

- Direct application of the drug into the affected zone (organ, tissue);
- Passive accumulation of the drug through leaky vasculature (tumors, infarcts, inflammation);
- Physical targeting based on abnormal pH and/or temperature in the target zone, such as tumor or inflammation (pH- and temperature-sensitive drug carriers);
- Magnetic targeting of drugs attached to paramagnetic carriers under the action of external magnetic field;
- Use of vector molecules possessing high specific affinity toward the affected zone. In a certain sense, cases (c) and (d) can be considered together as a 'physical targeting'.

The goal of the drug delivery systems is to put the medications to particular parts of the body by means of

either a physiological or a chemical trigger, such as "smart" drug carriers. The smart drug carrier is synthesized by polymer, which can carry or release drugs *via* in response to physiological conditions or external stimuli such as pH, temperature, magnetic or electric field. Pharmaceutical carriers include soluble polymers, nanocapsules, nanoparticles, cells, cell ghosts, lipoproteins, liposomes, and micelles. All of them can be made targeted in one way or another. The recognition of the target can occur:

- On the level of a whole organ.
- On the level of certain cells specific for a given organ.
- On the level of individual components characteristic of these cells, such as cell surface antigens.

Until recently, cancer treatment was largely based on the location in the body where the tumor began, such as the lung or breast. Now, cancer treatment increasingly depends on specific factors of a person's tumor, such as gene mutations (changes) or proteins that are often characteristic of cancer cells, regardless of the original location of the cancer. A treatment that targets these faulty genes or proteins that contribute to cancer growth and development is called a targeted treatment. Unlike previous generations of cancer chemotherapies that were developed to interfere with cancer cells as they divide into new cancer cells, a targeted treatment is designed to turn off a signal that tells cells to divide or delay cell death.

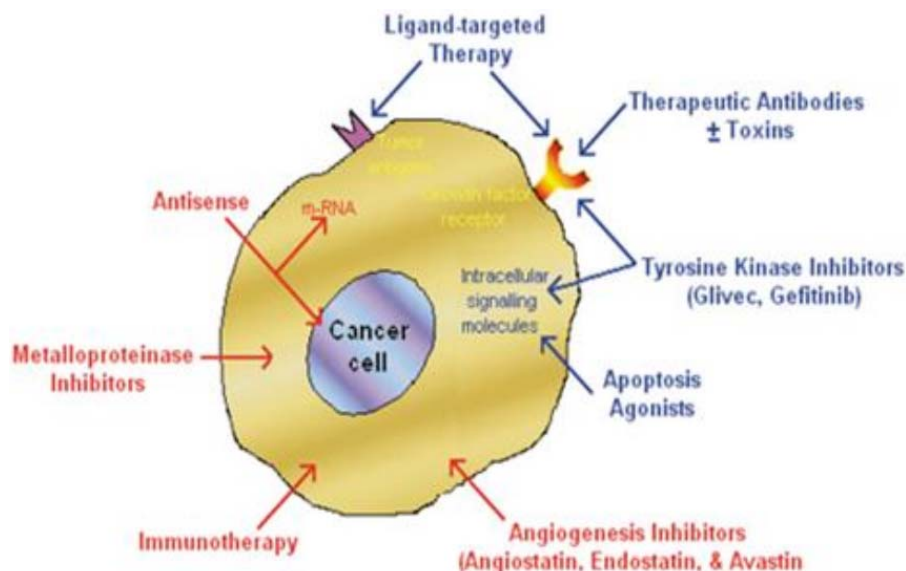


Figure 3: Different types of targeted therapies.

6.8.2. Principle Involved in Cancer and Tumor Targeting

The first formal statement of its primary goals made by the US Food and Drug Administration (FDA) suggests that a targeted-action drug is understood to be a drug whose prescription has to be preceded by a formally registered diagnostic test proving the existence of a target for its effect. It is required that this diagnostic test be an integral part of an implicit combination, of which a targeted-action drug is a component, and, though supplied under different covers, must always be used as a single whole in order to provide the maximum therapeutic effect [42]. Although this definition provides a comprehensive description of one of the basic principles of targeted therapy, i.e. identification of a target prior to its prescription to a patient (or, more correctly, to patients whose tumors express this target). The main requirements for targeted therapy are as follows:

- validated effect on the target (receptor, growth factor, etc.) critical for tumor survival and not compromising for normal organs and tissues;
- validation and estimation of a target(s) by registered diagnostic tests; prediction of efficiency of anticancer therapy (e.g. high efficiency in the presence of a target and zero efficiency in its absence);
- lack of toxicity related to the basic mechanism of the drug action and low or zero level of nonspecific toxicity (allergic reactions, coagulation effects, etc.).

The use of this set of criteria helps overcome the main disadvantages of conventional chemotherapy—empirical prescription of drugs (the same chemotherapy is usually prescribed to all patients with a definite type and stage of tumor without their preliminary allocation into cohorts with the highest prognostic beneficial therapeutic effect) and their nonspecific effect (the use of drugs in maximally effective doses is often limited by their general toxicity).

At present, a vast array of antitumor agents “claimed” as targeted-action drugs by manufacturers and “accepted” for use by practitioners in the field have undergone registration and/or are undergoing clinical trials. The most popular of them are as follows:

- drugs acting on receptors (and ligands thereof), enzymes, etc., mediating signal transmission to tumor cells (antibodies, small molecules, etc.);

- drugs inhibiting tumor microenvironment critical for tumor survival;
- antibodies eliciting immune responses and/or delivering toxic substances (radioactive materials, cytostatic drugs, etc.) to tumor cells.

Most of these agents are devoid of cytotoxic activity and ability to kill or damage tumor cells, but exert pronounced cytostatic effects by inhibiting proliferation and/or stimulating differentiation of tumor cells through inhibition of mechanisms responsible for the formation of the malignant phenotype.

The best drugs are those that can perform the desired function at the lowest dose possible, with the fewest side effects. Rational drug design depends on identifying a biomolecule (such as a protein) that causes disease and then tailoring a drug to alter or inhibit the function of that protein. The Figure 4 describes one scenario for the rational design of a chemotherapeutic agent. First, tissue from a healthy brain and from a cancerous brain tumor is collected, and the proteins from each sample are extracted. The proteomes of both samples are then analyzed *via* two-dimensional gel electrophoresis, which separates the proteins by size in one dimension, and by electrical charge in the second dimension. Comparison of the resulting pattern of protein spots results in the identification of a protein that is solely present or present to a much larger degree in the cancerous tissue. This protein is carefully collected and purified, and its three-dimensional structure is then determined *via* X-ray crystallography.

The structural information is used to design compounds that will bind to the protein’s active site. In the final steps of the design, the most promising compounds are synthesized and tested to ensure that they have the desired effect of halting the growth of the cancerous tissue, without unacceptable side effects.

6.8.3. Mechanisms of Targeted Cancer Therapies

Targeted cancer therapies interfere with cancer cell growth and division in different ways and at various points during the development, growth, and spread of cancer. Many of these therapies focus on proteins that are involved in the signaling process. By blocking the signals that tell cancer cells to grow and divide uncontrollably, targeted cancer therapies can help to stop the growth and division of cancer cells.

Targeted cancer therapies include several types of drugs. “Small-molecule” drugs block specific enzymes

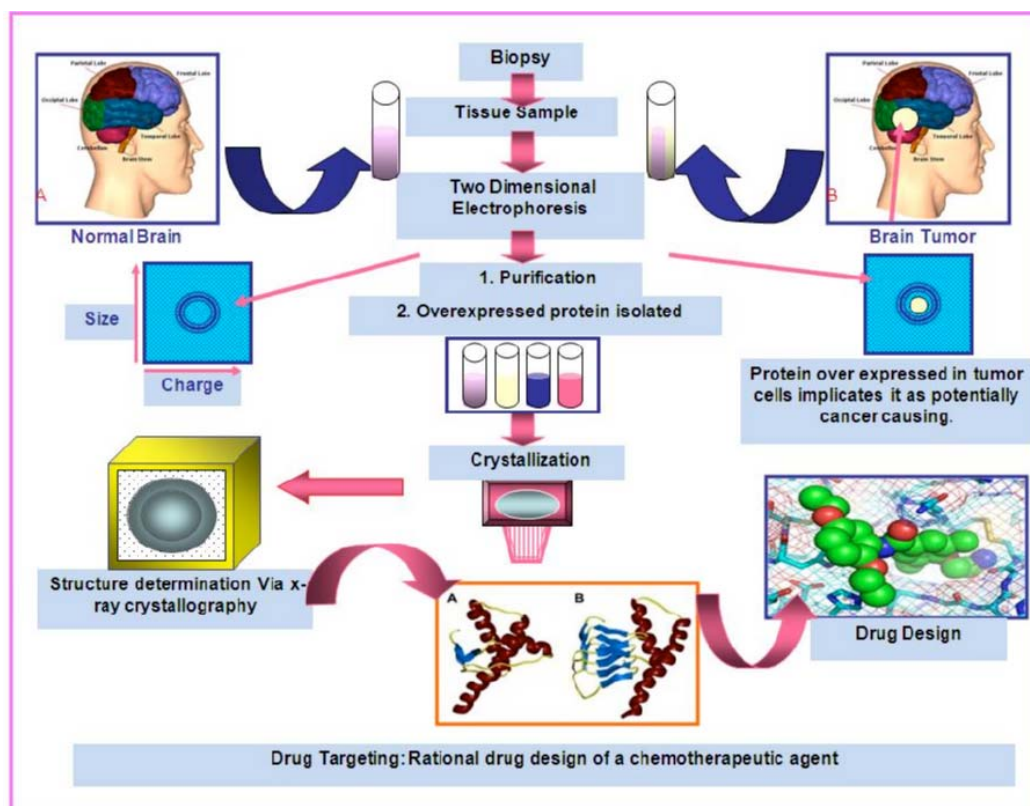


Figure 4: Drug targeting: Rational drug design of a chemotherapeutic agent.

and GFRs involved in cancer cell growth. These drugs are also called signal-transduction inhibitors. The small receptor site on the outside of a cell that is designed to have a signal protein attaches to it, which when attached signals the cell to duplicate. If a drug is able to block that receptor site on the surface of a cancerous cell, when the signal protein comes to tell it to replicate it will not be able to deliver the message. This effectively stops that cancer cell from duplicating into additional malignant cells. "Apoptosis-inducing drugs" cause cancer cells to undergo apoptosis (normal programmed cell death) by interfering with proteins involved in the process. When the gene which controls this process is altered, the cell becomes immortal. These types of drugs turn that cell death function back on so that apoptosis will take place, allowing the cancerous cell to die. "Angiogenesis inhibitors" may prevent the formation of blood vessels. In anticancer therapy, an angiogenesis inhibitor prevents the growth of blood vessels from surrounding tissue to a solid tumor, essentially starving it to death and preventing it from creating new pathways to spread through the circulatory system to other parts of the body.

6.8.4. Nanotechnology and Targeted Therapy

Nanotechnology has the potential to revolutionize cancer diagnosis and therapy. Advances in protein

engineering and materials science have contributed to novel nanoscale targeting approaches that may bring new hope to cancer patients. Nanocarriers encounter mucosal barriers and non-specific uptake barriers en route to their target. One way to overcome these limitations of nanocarriers by programming by a variety of conjugation chemistries such as by attaching targeting agents (ligands — molecules) so they actively bind to specific cells after extravasation. However, to date, there are only a few clinically approved nanocarriers that incorporate molecules to selectively bind and target cancer cells [43]. The potential of drug targeting was first suggested by the visionary Paul Ehrlich in 1906 [44]. Nanotechnology opens a new bright window in molecular oncology and promises to reformulate new and preexisting drugs into nanoparticles with altered biodistribution and improved pharmacokinetics; the goal is to reduce side effects, to enhance therapeutic efficacy by targeting the nanoparticles to tumors and by hiding the drug from plasma proteins or macrophages to ensure longevity in body fluids, essential for targeting to tumors using the leakiness of their vasculature. One fruit of the effort evolved from the genesis of the field of liposomes [45]. Nanoparticles composed of a variety of polymers including peptides, dendrimers, hyperbranched polymers, polyethyleneimine, poly (lactide-co-

glycolide), chitosan, metallic nanoparticles [46], colloidal drug delivery systems [47], and magnetic nanoparticles for cancer imaging have been developed. Paclitaxel, doxorubicin, 5-fluorouracil and dexamethasone have been successfully formulated into nanomaterials whereas nanodiamond hydrogels were used for doxorubicin formulations [48]. Surface modification of nanoparticles permits the attachment of targeting molecules for therapeutic applications [49]. Tumor specific drug targeting involving liposomes, immunoliposomes, microspheres and nanoparticles are now widely used at the experimental level and several have entered the clinic [50]. A schematic comparison of untargeted and targeted drug delivery systems is shown in Figure 5.

7. STRATEGIES OF TARGETING

The primary requirements for the development of effective targeting therapeutic modalities for the treatment of cancer are the tumor-targeted delivery of the therapeutic molecules of interest to the tumor site(s) in the body (both primary and metastatic), passage of the molecular therapeutic through the cell membrane, and targeting specifically a growth or apoptotic pathway [51]. Ideally, for anticancer drugs to

be effective in cancer treatment, they should first, after administration, be able to reach the desired tumor tissues through the penetration of barriers in the body with minimal loss of their volume or activity in the blood circulation [52]. Second, after reaching the tumor tissue, drugs should have the ability to selectively kill tumor cells without affecting normal cells with a controlled release mechanism of the active form. These two basic strategies are also associated with improvements in patient survival and quality of life by increasing the intracellular concentration of drugs and reducing dose-limiting toxicities simultaneously. The three general strategies of targeting include the passive targeting of bulk cancerous tissue, where the body concentrates inert nanoparticles or by active targeting of unique tumor cells, where functional modification of the surface of the particle enhances the therapeutic delivery system resulting in specific tissue targeting [53], and physical targeting. The all the three approaches are illustrated in Figure 6.

7.1. Passive Targeting

Passive targeting relies on the properties of the delivery system and the disease pathology in order to preferentially accumulate the drug at the site of interest

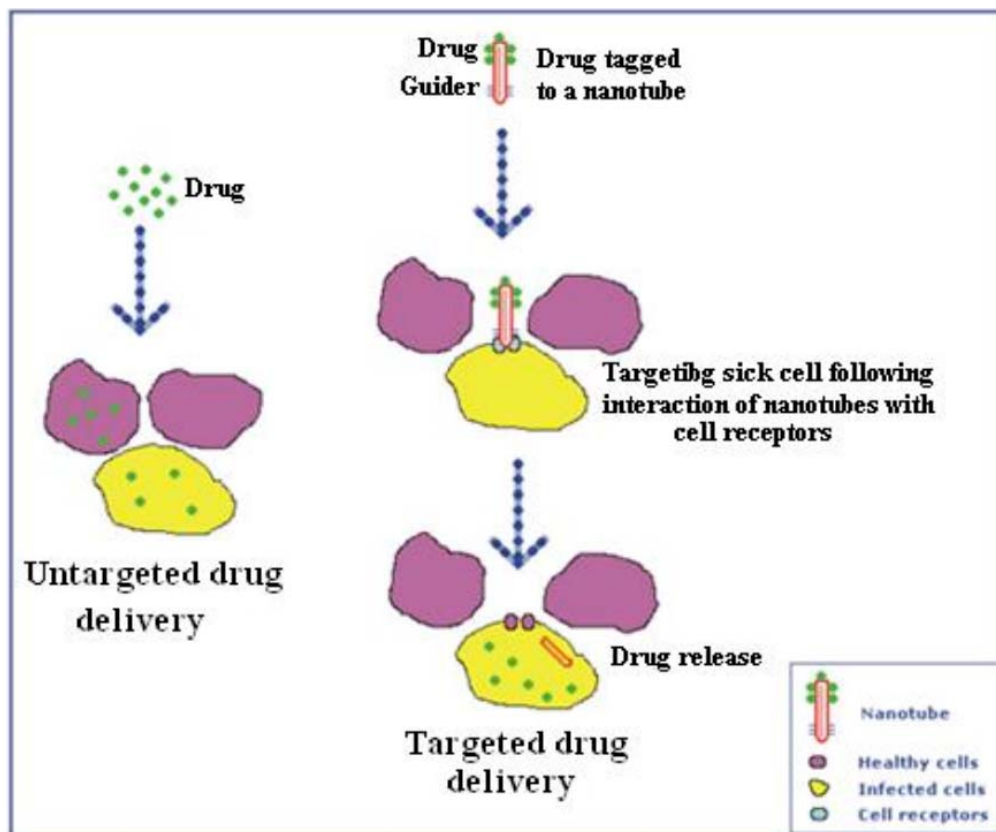


Figure 5: Difference between targeted and non-targeted therapies.

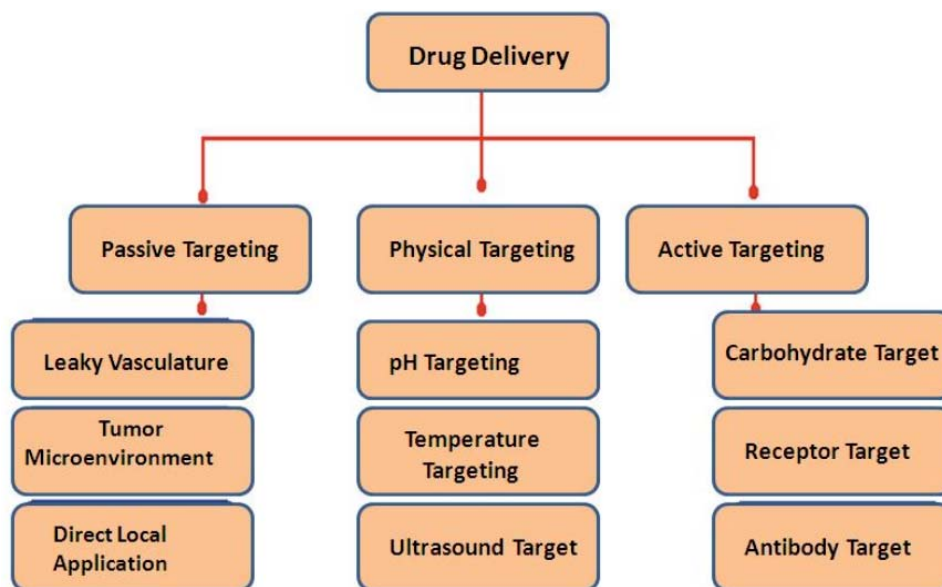


Figure 6: Targeting mechanisms of nanoparticles drug delivery.

and avoid non-specific distribution. Passive targeting takes advantage of the permeability of tumor tissue. Rapid vascularization to serve fast-growing cancerous tissue lends itself to a leaky and defective architecture, which in turn, can be easily accessible to toxic chemotherapeutic drugs. Some drugs can be administered as prodrugs or inactive drugs, which once exposed to the tumor environment, can be switched on to become highly active. Passive targeting also incorporates the delivery of drug to the tumor bed through several invasive modalities.

7.1.1. Leaky Vasculature

Most polymer nanoparticles display the enhanced permeability and retention effect (Figure 7). First described by Maeda *et al.* [54], the enhanced permeability and retention phenomenon is based on two factors: (a) the capillary endothelium in malignant tissue is more disorderly and thus more permeable towards macromolecules than the capillary endothelium in normal tissues. This allows extravasation of circulating polymeric nanoparticles within the tumor interstitium, and (b) the lack of tumor lymphatic drainage [55] in the tumor bed results in drug accumulation. If a chemotherapeutic agent is coupled to a suitable polymer or other molecular carrier *via* a degradable linker, then such carriers have the potential of increasing the concentration of the chemotherapeutic agent within the tumor tissue. As a result of these characteristics, concentrations of polymer-drug conjugates in tumor tissue can reach levels 10 to 100 times higher than that resulting from the administration of the free drug [56].

Other factors which influence EPR include the size of tumors, degree of tumor vascularization, and angiogenesis. Usually, less nanoparticle accumulation is seen in preangiogenic or necrotic tumors. Tumoral angiogenesis means the sprouting of new blood vessels from existing vessels. Development of new blood vessels is an important event in tumor progression, since it supports tumor growth and allows the dissemination of cancer cells throughout the body, leading to metastasis. Pathological angiogenesis has been implicated in over 20 other diseases, including obesity [57], asthma, diabetes, and multiple sclerosis.

Further approaches for passive targeting involve size of the nanocarriers and surface charge modulation. The optimum size of nanoparticles that can be accumulated in a tumor by the EPR effect is not yet precisely known. However, studies using liposomes and nanoparticles have indicated that the cutoff size of the pores in tumor vessels is as large as 200nm to 1.2 mm [58] and direct observation of tumor vasculature has demonstrated a tumor dependent pore cutoff size ranging from 200 nm to 2 mm [59]. These size ranges seem to indicate that drug-loaded nanoparticles may be accumulated in malignant tumor cells. Polymer-based nanoparticles bearing DOX were found to circulate in the blood for more than 3 days, and gradually accumulated in tumors *via* the EPR effect [60].

These days, antiangiogenic therapy is considered one of the most promising approaches for eradicating cancer. Various strategies for interrupting the

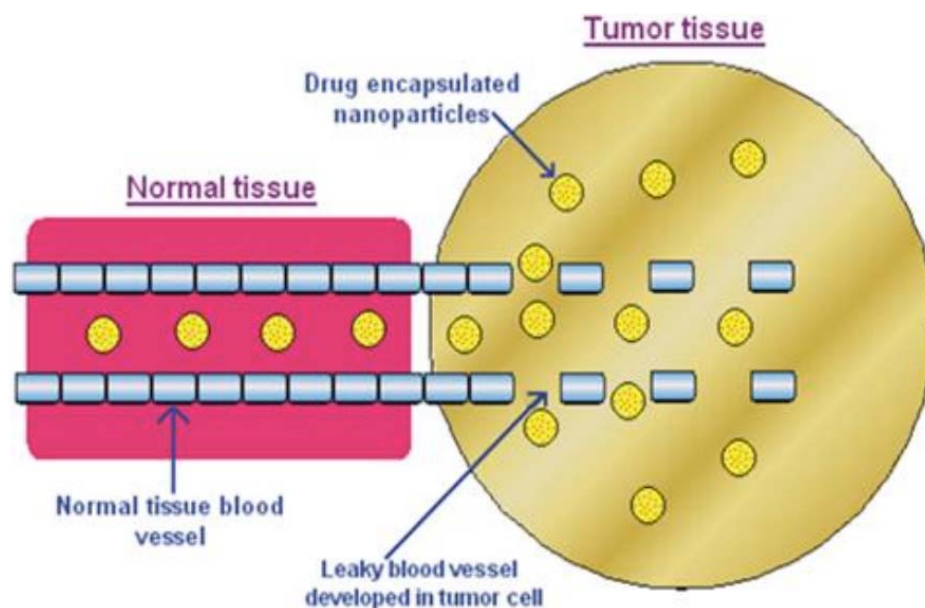


Figure 7: Schematic diagram of nanoparticles accumulation in tumor tissue *via* EPR effect: Passive targeting.

angiogenic process have been investigated, including inhibition of the endogenous angiogenic factors (e.g. growth factors), degradative enzymes (e.g. MMPs), and endothelial cell processes (e.g. differentiation, activation, migration, and proliferation) necessary for angiogenesis. The tumor vasculature generated by angiogenesis in cancer is morphologically abnormal, and various cell-surface proteins have been associated with promoting angiogenesis. Thus, it should be possible to selectively destroy tumor neovasculature without significantly affecting normal vessels.

7.1.2. Tumor Microenvironment

Another contributor to passive targeting is the unique microenvironment surrounding tumor cells, which is different from that of normal cells. The drug is conjugated to a tumor-specific molecule and is administered in an active state, and once it reaches its destination, the tumor environment is able to convert it to an active and volatile substance, so-called tumor-activated prodrug therapy. Fast-growing, hyperproliferative cancer cells show a high metabolic rate, and the supply of oxygen and nutrients is usually not sufficient for them to maintain this. Therefore, tumor cells use glycolysis to obtain extra energy, resulting in an acidic environment [61]. The pH-sensitive liposomes are designed to be stable at a physiologic pH of 7.4, but degraded to release active drug in target tissues in which the pH is less than physiological values, such as in the acidic environment of tumor cells [62].

Additionally, cancer cells express and release unique enzymes such as matrix metalloproteinases,

which are implicated in their movement and survival mechanisms [63]. Mansour *et al.* [64] developed a water-soluble maleimide derivative of doxorubicin, incorporating a matrix metalloproteinase-2-specific peptide sequence. This polymer-drug conjugate had a high affinity for the cysteine-34 position of circulating albumin. The albumin-bound form was efficiently cleaved by the matrix metalloproteinase-2 liberating free doxorubicin.

7.1.3. Local Drug Administration

Direct local application allows the drug to be given directly to tumor tissue, avoiding systemic circulation. Various approaches have been taken to improve the tumor delivery of anticancer agents, such as intravesical injection and i.p. administration of various agents. These approaches require exposure to higher concentrations of antitumor agents, which is not always feasible. Localized drug delivery through intratumoral administration is an attractive approach that has been tried and tested [65]. The administration of mitomycin directly into tumor tissue resulted in an increased concentration of the drug at the tumor site and decreased toxicity. It involves direct delivery of the drug to a localized tumor site, thus excluding the systemic side effects of the drugs, while concentrating drug levels at their site of action. However, not all tumor types are amenable to such an approach, for example, lung cancer. However, for prostate cancer treatment such an approach can be effective. Recently it has been shown that direct intratumoral delivery of paclitaxel in biodegradable nanoparticles, which were

conjugated to transferrin (Tf) ligand, demonstrated complete tumor regression in subcutaneous mice model of prostate carcinoma [66]. The mechanism of greater efficacy of Tf-conjugated nanoparticles was determined to be due to greater cellular uptake and sustained intracellular retention of the encapsulated drug than that with drug in solution or unconjugated nanoparticles.

7.2. Active Targeting

Active targeting to the disease site relies on addition to PEG modification of nanocarriers to enhance circulation time and achieve passive targeting coupling of a specific ligand on the surface that will be recognized by the cells present at the disease site [67]. Active targeting requires the therapeutic agent to be achieved by conjugating the therapeutic agent or carrier system to a tissue or cell-specific ligand [68]. Different types of nanoparticles are developed to deliver drug at target site (Figure 8). These ligands are extraordinary in that they can recognize and bind to complementary molecules, or receptors, found on the surface of tumor cells. When such targeting molecules are added to drug delivery nanoparticles, more of the anticancer drug finds and enters the tumor cell, increasing the efficiency of the treatment and reducing toxic effects on surrounding normal tissue [69].

Active targeting can be achieved by the functionalization of NPs with ligands such as antibodies, peptides, nucleic acid aptamers, carbohydrates, and small molecules.

7.2.1. Monoclonal Antibodies

Antibodies are the first macromolecular ligands used for targeted delivery. The use of monoclonal antibodies (mAb) became widespread after the discovery of hybridoma technology. Due to their inherent immunogenicity, murine monoclonal antibodies were not suitable for clinical applications. Engineering antibody technologies led to the development of chimeric humanized and fully humanized antibodies. More recently, methods have been developed to produce human immunoglobulins from transgenic mice. Combinatorial phage display libraries have also emerged as a powerful tool to select novel protein ligands [70]. The latter approach relies on multiple selection and screening parameters to select human antibodies against specific cell types or antigens. The selection process can be designed to improve the properties of the ligand such as stability, affinity, selectivity and internalization. Most recently, this approach was used to isolate cancer targeting antibodies using live cancer patients in the selection process [68, 71].

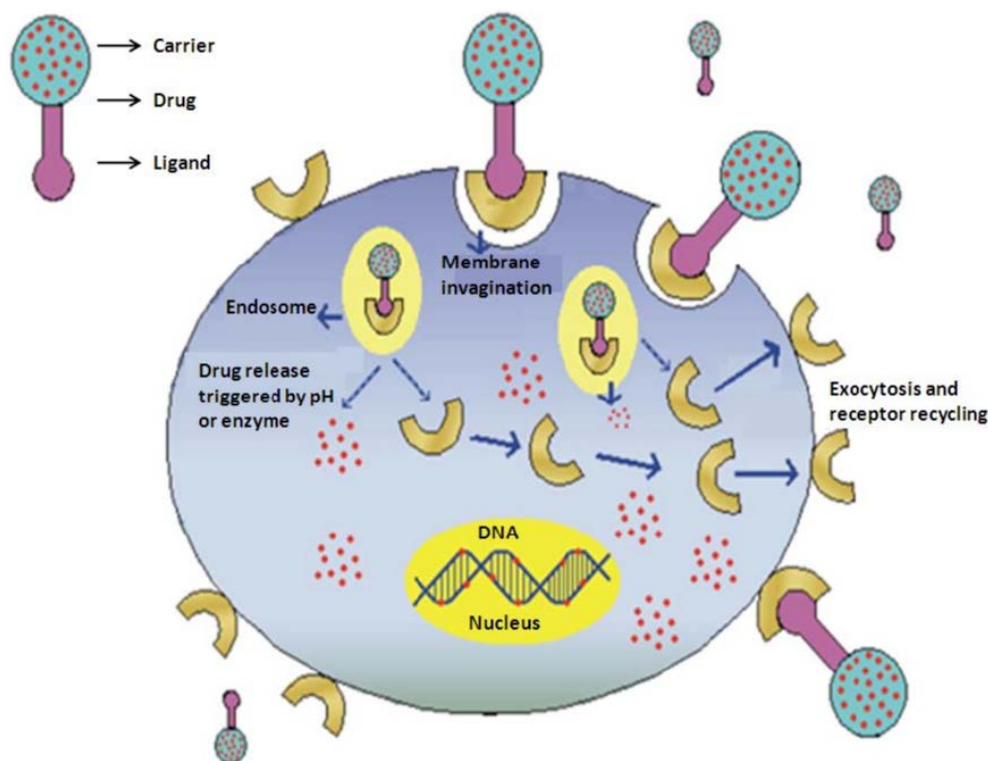


Figure 8: Active targeting: internalization of nanoparticles *via* receptor mediated endocytosis.

Antibody fragments show less immune response and can be stabilized with disulfide bond or chemically crosslinked. These antibodies function by antagonizing the signal transduction pathway that leads to uncontrolled growth in cancer cells. Recent investigations have focused on exploiting the affinity of these antibodies for their respective antigens to “arm” them with chemotherapy drugs (doxorubicin, methotrexate, and calicheamicin), toxins, or radionucleotides [72].

7.2.2. Aptamer Based Targeting Molecule

Nucleic acid ligands are analogue of antibodies and they can bind with high affinity and specificity to a broad range of targets, such as small molecules, proteins, viruses, or cells, also known as aptamers, are a class of macromolecules that are isolated from combinatorial libraries of synthetic nucleic acid by an iterative process of adsorption, recovery and reamplification and can be generated against amino acids, drugs, proteins and other molecules. Single-stranded RNAs which can recognize target molecules through folding into specific 3-dimensional structures are termed RNA aptamers. [73-75]. Nucleic acid aptamers are single stranded DNA, RNA or unnatural oligonucleotides that fold into unique structures capable of binding to specific targets with high affinity and specificity [76-77]. As small molecules, with a half-life of minutes to hours due to nuclease degradation, aptamers can be rapidly cleaned from the bloodstream by the kidneys [78]. The combination of aptamers with novel nanomaterials, including nanomaterial-based aptamer bioconjugates has attracted considerable interest and has led to a wide variety of applications. Bagalkot *et al.* [79] have also demonstrated a novel strategy for the targeted delivery of anthracyclic agents including doxorubicin directly to cancer cells through the formation of an aptamer doxorubicin physical conjugate.

Recently, Farokhzad *et al.* [80] have elegantly described the use of aptamers, nucleic acid constructs that specifically recognize prostate membrane antigen on prostate cancer cells. The aptamer technology provides an additional strategy for active targeting to tumor cells in the body using a monoclonal antibody, 2C5, which specifically recognizes antinuclear histones.

7.2.3. Oligopeptide-Based Targeting Molecules

Peptides are small, synthetic molecules that can be manufactured in large quantities with excellent quality

control. Peptides are more stable than antibodies and unlikely to be immunogenic. The discovery of new peptide targeting domains has been successful due to the development of peptide library screening methods (e.g., phage display; $\sim 10^{11}$ different peptides) [81]. Peptides have gained a lot of attention as a potent targeting ligand.

Peptides are becoming an attractive alternative to antibodies because of their small size, lower immunogenicity, higher stability, and ease of manufacture. Recent development of phage display screening methods has successfully isolated peptide ligands with high specificity and affinity to cell-surface hormone receptors (LHRH receptors, somatostatin receptors [82] and tumor vasculature antigens.

7.2.4. Folate-Based Targeting Molecules

One of the most extensively studied small molecule targeting moieties for drug delivery is folic acid (folate). The high-affinity vitamin is a commonly used ligand for cancer targeting because folate receptors (FRs) are frequently over-expressed in a range of tumor cells. Folate specifically binds to FRs with a high affinity ($KD = \sim 10^{-9}$ M), enabling a variety of folate derivatives and conjugates to deliver molecular complexes to cancer cells without causing harm to normal cells. It has been used as a targeting moiety combined with a wide array of drug delivery vehicles including liposomes, protein toxins, polymeric NPs, linear polymers, and dendrimers to deliver drugs selectively into cancer cells using FR-mediated endocytosis.

Recently, dendrimer-based targeted anticancer therapeutics using folate have demonstrated promising *in vivo* efficacy in terms of targeting and specific killing of cancer cells *via* multivalent interaction [83]. When the surface of the nanocarriers is modified with folic acid, they can be targeted to the tumor cells that overexpress folate receptors. The nanoparticle conjugated with folic acid as a receptor seeker and containing methotrexate as a chemotherapeutic agent has been demonstrated to enter the cancer cell and inhibit its growth [84].

7.3. Physical Targeting

It is a new targeting strategy known as physical targeting, which makes use of an external stimulus to target the release of drug at a specific site in the body (Figure 9). The physical properties, such as swelling/deswelling, particle disruption and aggregation

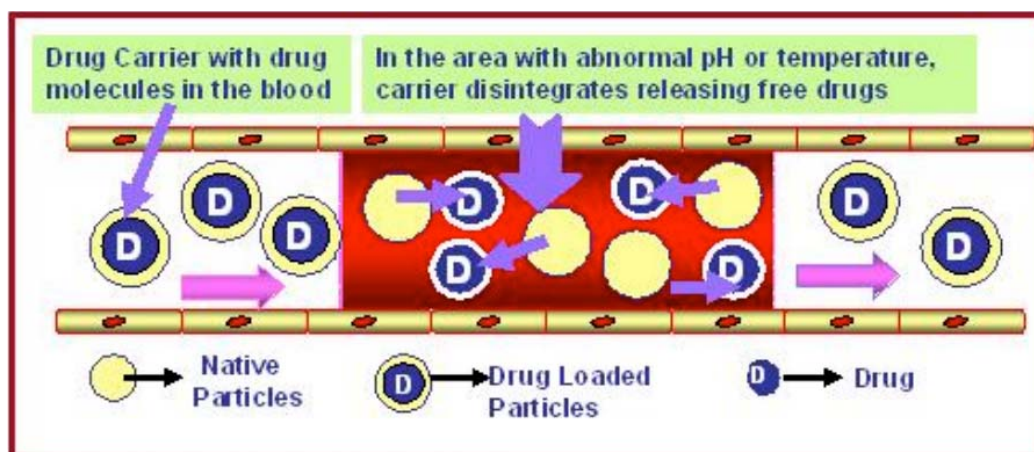


Figure 9: Physical targeting of drugs.

of stimuli-responsive nanocarriers change with respect to changes in environmental conditions.

In turn, these properties alter the interactions of the nanocarriers with the cells and trigger the drug release from slow to fast at the tumor site. Various physical targeting strategies are as follows:-

7.3.1. pH Sensitive Targeting

More recently, pH-sensitive polymeric carriers have been used in targeted antitumor drug delivery [85], based on intrinsic differences between various solid tumors and the surrounding normal tissues in terms of their relative acidity [86]. The extracellular pH (pHe) in most tumors is more acidic (pH 5.7-7.4) than in normal tissues [87]. Change of pH at tumor tissue is useful, because tumor tissues have a more acidic environment, due to lactic acid produced by hypoxia and by acidic intracellular organelles [88]. Therefore, various pH-responsive polymeric nanoparticles have been used for rapid anticancer drug release at tumor sites.

Anticancer drugs can be conjugated to pH-sensitive polymers to exploit the acidic environment of tumor. Presence of acid sensitive spacers between the drug and polymer enables release of drug either in relatively acidic extracellular fluids or, after endocytosis in endosomes or lysosomes of tumor cells. Kamada and colleagues [89] synthesized a pH-sensitive polymeric carrier, in which a poly(vinyl pyrrolidone-co-dimethyl maleic anhydride) (PVD) was conjugated to doxorubicin (DOX), that could gradually release free drug in response to changes in pH [i.e., from near neutral (~7.0) to slightly acidic pH (~6.0)].

In their recent work, Bae and co-workers have reported the intracellular pH-sensitive polymeric

micelles that can release the anticancer drug, DOX, in response to acidic pH at endosomes (pH 5.0–6.0) and lysosomes (pH 4.0–5.0), which can maximize the DOX delivery efficiency to the tumor tissue [90].

7.3.2. Temperature Sensitive Targeting

Cancer cells are very sensitive to elevated levels of heat as cancer cells die and tumor sizes reduce upon exposure to prolonged heat, temperatures between 40-46°C. The prolonged heating of the tissue in the range of 40-46°C is known as hyperthermia [91], and it has been used for treatments of various cancer diseases. Different mechanisms such as protein coagulation, membrane fluidity, and nucleic acid modifications might be responsible for the cancer killing cells in hyperthermia. Temperature sensitive nanoparticles [92, 93] are a potential candidate for inducing drug release in combination with hyperthermia therapy. After intravenous injection, these particles can be targeted to the cancerous tissue. Heat provided in hyperthermia therapy would, in turn, act as a stimulus to the particles to release the drugs to the surrounding tissue. Hyperthermia is reported to increase tumor blood flow and tumor vascular permeability compared to normal vasculature [94]. Hyperthermia also makes cancer cells more sensitive to anticancer therapies including anticancer drugs. PNIPA particles would release anticancer drugs at these temperatures causing a significant reduction in the tumor size due to the combination of hyperthermia and drug activity.

Chilkoti *et al.* [95] in their study reported that a thermally responsive ELP exhibits a 2-fold greater accumulation in solid tumors that are heated to 42 °C and ~2-fold increase in cellular uptake under hyperthermic conditions when compared with an identical ELP under normothermia.

7.2.3. Ultrasound Sensitive Targeting

Ultrasound is a safe, portable, and low-cost imaging modality that shows excellent potential for applications in molecular imaging and targeted drug delivery [96]. Ultrasound (US) has an ever-increasing role in the delivery of therapeutic agents including genetic material, proteins, and chemotherapeutic agents. Ultrasound can “see” into the body (e.g., diagnostic ultrasound) and can be used to transmit energy into the body at precise locations. This safe, non-invasive and painless transmission of energy into the body is the key to ultrasonic-activated drug delivery. Major mechanisms that are involved in ultrasound-triggered drug release are local temperature increase [hyperthermia], cavitation which increases the permeability of cell membranes, and the production of highly reactive free radical species which can accelerate polymer degradation [97].

Howard et al. [98] studied the effect of ultrasound on paclitaxel in micelles of methyl capped poly(ethylene oxide)-co-poly-(L-lactide)-tocopherol on a breast cancer drug-resistant cell line. Their study used 1 MHz ultrasound at a power density of 1.7 W/cm² and duty cycle of 33%. They showed that upon the application of ultrasound, drug accumulation from encapsulated Paclitaxel drastically increased and surpassed the amount of drug found in non-irradiated cells.

Juffermans and his colleagues [99] studied ultrasound and microbubble-targeted delivery which provides opportunities for new therapies due to its low toxicity, low immunogenicity, noninvasive nature, local application and its cost-effectiveness. The bio-effects found in their studies provide important new insights into the mechanisms of ultrasound and microbubble-targeted delivery of therapeutic compounds and will lead to the rational design of new drug or gene therapies involving ultrasound and microbubbles (Figure 10).

7.3.4. Magnetically Sensitive Targeting

Magnetism has a profound influence on living organisms. It is an established fact that the magnetism and magnetic materials have a strong role to play in health care and biological applications [100]. Magnetic carriers receive their magnetic response to a magnetic field from incorporated materials such as magnetite, iron, nickel, cobalt etc. Magnetic particles functionalized with the drug can serve as potential drug carriers in a new drug delivery strategy based on the application of external magnetic fields. The principle of drug delivery by magnetic carrier is based on the use of both constant and high-frequency oscillating magnetic fields. The carriers typically have one of two structural configurations: (i) a magnetic particle core (usually magnetite, Fe₃O₄, or maghemite, γ -Fe₂O₃) coated with a biocompatible polymer or (ii) a porous biocompatible

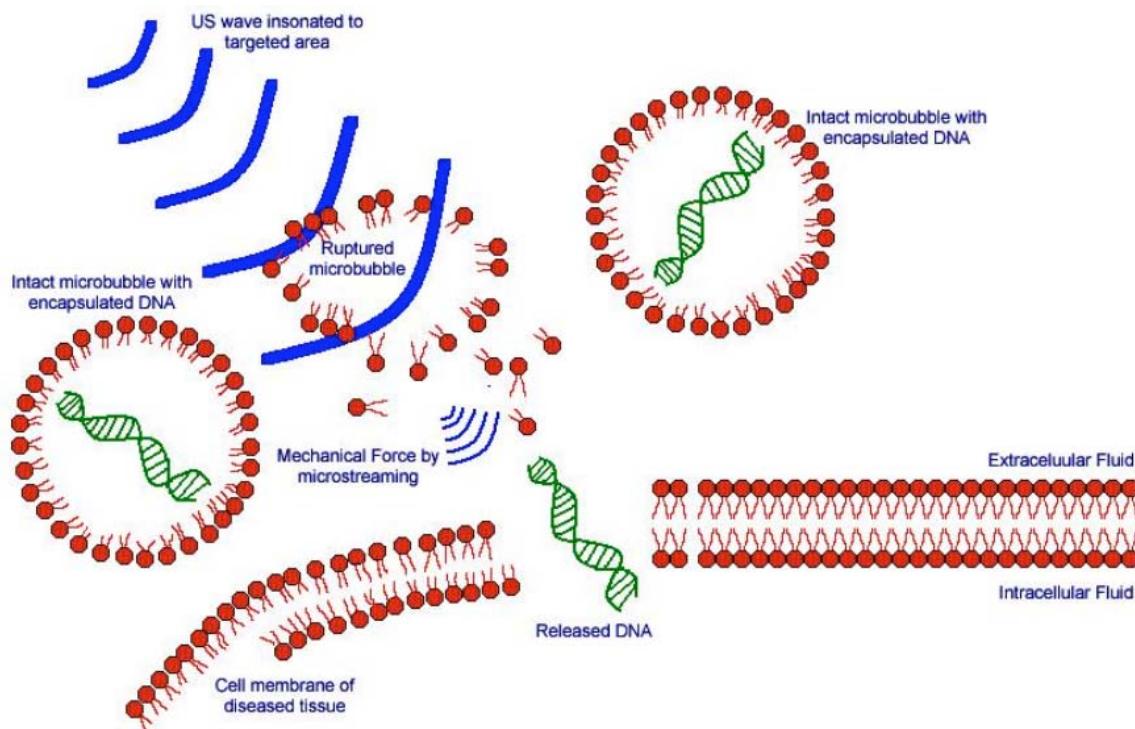


Figure 10: Ultrasound microbubble-targeted drug delivery.

polymer in which magnetic nanoparticles are precipitated inside the pores [101]. A constant magnetic field provides targeted drug delivery, while a high-frequency oscillating magnetic field is responsible for the controlled release of encapsulated drug. Since the magnetic particles are attracted to high magnetic flux density and thus can be targeted to specific areas (cancer tissues) by external constant magnetic fields (Figure 11). Releasing mechanism is evoked by thermal excitations of these biocompatible magnetic particles induced by an external high-oscillating magnetic field [102].

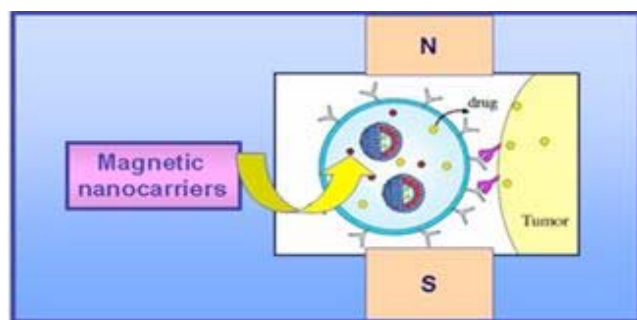


Figure 11: Example of tumor targeting in the presence of magnetic field.

Magnetic release can control the activity of a drug delivery system in several ways, including decreasing the amount of unnecessary damage to healthy tissue, increasing the efficacy of the drug, and treating ailments in a minimally invasive way. Unnecessary damage to healthy tissue can be decreased by using an external magnet to guide polymeric particles containing magnetic material and drugs to cells that need to be treated; [103] an external magnetic field can then be used to release the drugs at the target site [104]. The hypothesis of magnetically induced drug release from polymeric, magnetic particles is that heat produced locally by magnetic particles in AC magnetic fields loosens the polymer strands surrounding the particles at temperatures above the glass transition temperature (T_g) of that particular polymer, allowing the encapsulated constituent to be released. Very high concentrations of chemotherapeutic or radiological agents can be achieved near the target site, such as a tumor, without any toxic effects to normal surrounding tissue or to the whole. The combination of these two advantages makes successful the magnetic nanoparticulate applications also in molecular and cell biology, advancing both basic science and clinical practice [105]. Also, magnetic fields are well suited for biological applications as they are not screened by biological fluids and do not interfere with most biological processes [106].

In magnetically targeted therapy, (Figure 12) a cytotoxic drug is attached to a biocompatible magnetic nanoparticle carrier. These drug carriers are injected into the patient *via* the circulatory system. When the particles have entered the blood stream, external, high-gradient magnetic fields are used to concentrate the drug carriers at a specific target site within the body. Employing magnetic induced delivery systemic chemotherapy could be effectively made to control the primary tumour without significant side effects, due to the targeting of magnetic liposomes encapsulated with anticancer drug adriamycin (ADR) [107]. Another *in vivo* study with doxorubicin showed that administration of magnetoliposomes under an applied external magnetic field produced an approximately four times higher doxorubicin concentration in the tumor compared to the doxorubicin solution applied. These results suggest that systemic chemotherapy could effectively control the primary tumor without significant side effects due to the specific targeting of magnetic doxorubicin liposomes [108]. Novel dual-functional nanospheres composed of magnetic iron oxide nanoparticles embedded in a thermo-sensitive Pluronic F127 (F127) matrix were successfully synthesized by Liu *et al.* [109]. It was found that F127 showed a rapid structural change and the magnetic phase caused rapid heating after a short exposure to a high-frequency magnetic field. Considerable volume shrinkage of the nanospheres resulted in an instantaneous release of a drug, Doxorubicin (DOX) due to an intimate contact between the nanomagnet and F127, where an effective thermal and mechanical transfer between core and shell phases efficiently took place in the presence of the magnetic field.

Superparamagnetic nanoparticles exhibiting higher magnetization and good biocompatibility are of particular interest as magnetic drug targeting carriers for hyperthermia [110] and as contrast enhancement agents in magnetic resonance imaging [111]. Hyperthermia is the use of therapeutic heat to treat various cancers on and inside the body. The purpose of this anticancer therapy is to shrink and hopefully destroy cancer without harming noncancerous cells. Hyperthermia can be used on very small areas of the body to the entire body itself. Local hyperthermia refers to heating only one body area, usually where the tumor is located. The potential of hyperthermia as a treatment for cancer was first predicted following observations that several types of cancer cells were more sensitive to temperatures in excess of 41°C than their normal counterparts [112, 113]. Therefore, by increasing the

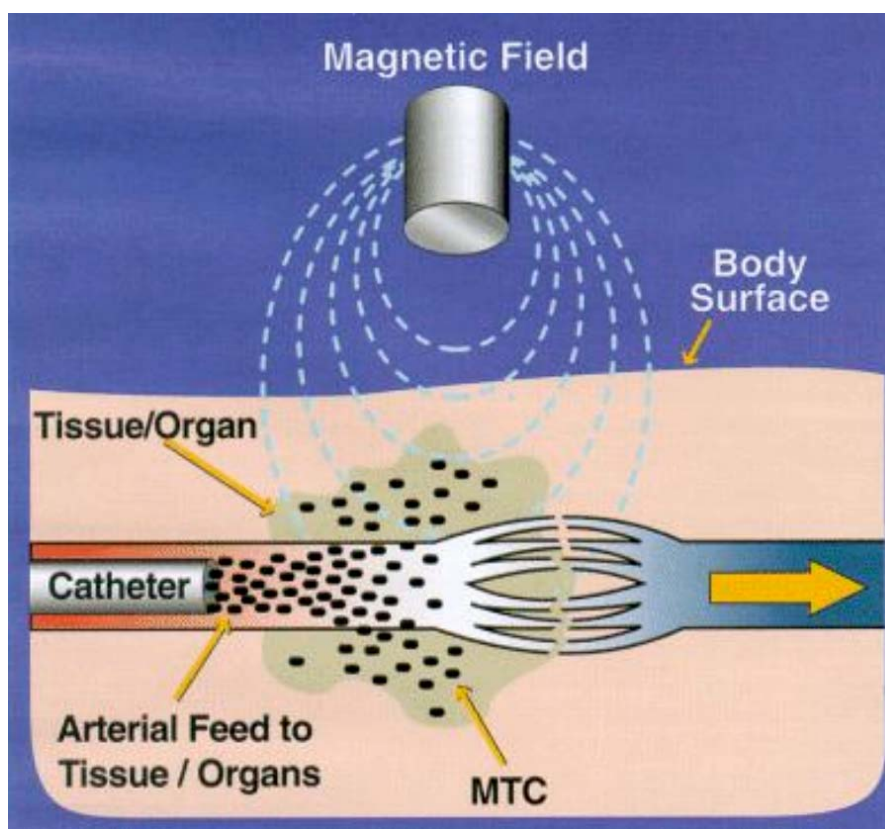


Figure 12: Magnetically targeted drug delivery by particulate carriers.

temperature of the tissue to more than 41°C, the cells could be selectively destroyed. To achieve this, a dose of superparamagnetic particles could be injected into a region of malignant tissue, after which an alternating magnetic field could be applied to the particles. If the field is sufficiently strong and of optimum frequency, the particles would absorb energy and heat the surrounding tissue, thereby, affecting only the infected cells. Hyperthermia is now being used more widely, because it does not have as many negative side effects as conventional forms of cancer treatment such as radiation or chemotherapy.

MRI is a common tool for diagnosis of malignant tumors based on the nuclear magnetic resonance of protons in the molecules, mainly water, that exist in a given tissue. Major advantage of MRI is its safety and ability to scan without radiation so that it does not destroy healthy cells in the human body. MRI became more useful for malignant tumour detection when assisted by contrast agents. The technique measures changes in the magnetization of hydrogen protons in water molecules sitting in a magnetic field after a pulse of radio frequencies has hit them. Protons from different tissues react differently, giving a picture of anatomical structures. These images can be enhanced

adding 'contrast agents' which sharpen the contrast by affecting the behavior of protons in their proximity [114]. Superparamagnetic nanostructured materials were developed as the contrast agents for MRI because the nanoscaled structure modified the relaxation time of protons and enhanced the sensitivity of diagnosis of MRI. Colloidal iron oxides therefore play an important role as MRI contrast agents, as superparamagnetic iron oxide particles were the first liver-specific contrast agents used [115]. It has been known for many years that the inclusion of magnetic particles within tissue enables a very large signal to be obtained from a MRI scanner. Therefore, for better imaging purposes, it would be useful to have a contrast agent that could target a specific tissue, organ, or tumor.

Brazel *et al.* [116] developed thermo-responsive polymers and found that it showed a self heating effect under AC magnetic field and can be used either for hyperthermia or to trigger the release of anticancer drugs. The CoFe_2O_4 nanoparticles dispersed in hexane were synthesized by a reduction technique. Kato *et al.* [117] published investigations into selective cancer chemotherapy, in which ferromagnetic mitomycin microcapsules (about 300 μm diameters) were magnetically guided to tumour sites of experimental

animals. The particles could be manipulated by fields of about 56 kA/μ. Jain *et al.* [118] developed a novel water-dispersible oleic acid pluronic-coated iron oxide nanoparticle formulation that could be loaded easily with high doses of water-insoluble anticancer agents. This nanoparticle formulation could be used as a universal drug carrier system for systemic administration of water-insoluble drugs while simultaneously allowing magnetic targeting and/or imaging.

8. COMMERCIALY AVAILABLE POLYMER BASED DRUG DELIVERY TECHNOLOGIES

Number of commercially available polymer mediated drug delivery systems has gained a pace in recent few years. This thrust is due to the fact that mode and mechanism of the delivery could be controlled by changing the composition of the macromolecules. Development in drug delivery technique has been grouped in three major categories. The first category covers all the drug delivery devices and systems, including biopolymers, drug carriers as well as pro-drug platforms. The second category consists of biological molecular approaches, which include recombinant proteins, antibody derivatives, peptides and oligonucleotide platforms including siRNA and aptamer technologies. Third and final category entails drug metabolism, pharmacokinetic/pharmacodynamic interactions [119, 120]. Global sincere efforts towards the development of innovative drug delivery materials deemed to improve the safety and efficacy of new or existing medicinal products. However, only a limited number of such technologies are commercially available for the clinical trials.

Zoladex depot[®] is a polymer based formulation of goserelin acetate dispersed in poly(lactic/glycolic acid) matrix [121, 122]. Goserelin acetate is an injectable gonadotropin releasing hormone superagonist Zoladex Depot[®] was approved by the U.S. Food and Drug Administration in 1989 for treatment of prostate cancer. Goserelin acetate suppress the production of the sex hormones testosterone and oestrogen, for the treatment of the breast and prostate cancer [123].

Lupron Depot[®] was the first commercial formulation consisting of leuprolide acetate and poly(lactic acid). Lupron Depot[®] represents a class of drugs that are known as gonadotropin releasing hormone analogs. It works by inhibiting the production of the hormone testosterone, which may play a significant role in the growth of prostate cancer. Decreasing the levels of testosterone in the body may also alleviate bone pain

and some urinary problems that may be associated with metastatic prostate cancer [124].

Sotiriou *et al.* [125] prepared silica-coated Ag-Fe₂O₃ nanoparticles with two faces using aerosol technology for drug delivery and imaging purposes. The advantages of silica coating are the reduction of toxic effects of silver and increased stability, with easy dispersion in biological solutions. As a summary, it can be said that trials are underway for an ultimate cure for cancer.

Sandostatin LAR[®] is a synthetic acetate salt of a long-acting cyclic octapeptide is highly prescribed and studied medical therapy in the treatment of acromegaly and gastroentero-pancreatic neuroendocrine tumors [126]. This drug is an injectable composition in a biodegradable copolymer of poly(glycolide) and poly(D,L-lactide).

Approximately 22 different siRNA/shRNA therapeutics have reached clinical testing for the treatment of at least 16 diseases. Marina Biotech has launched a Phase I trial of the first orally administered shRNA drug for treating familial adenomatous polyposis (FAP), a rare hereditary disease that often leads to colon cancer. The CEQ508 shRNA therapy down regulates β-catenin to slow the polyp growth in intestinal cells [127]. The drug is encapsulated by the company's TransKingdom RNA interference (tkRNAi) technology, which utilizes nonpathogenic *Escherichia coli* to produce and deliver the shRNAs to target cells [128].

9. CHALLENGES AND FUTURE SCOPE

Even after an adequate advancement of technology and understanding of the inside biology of cancer and tumor, the field of targeting delivery of drugs still lacks in perfection of treatment and patients satisfaction. Moreover, even at present the field needs more and more technological know how, effective drugs, precise targeting tools, economic viability and more research and development for better cure of cancer and tumor. Some of the specific points that demand attention of medical and science experts may be mentioned as below:

1. Precise and accurate targeting of the affected site of the tumor area.
2. Minimized loss of normal cells due to targeting.
3. Better guiding methods to take into consideration the patient's comfort, reduced span of

treatment period and effectiveness of the treatment.

4. Close monitoring of post targeting conditions of the patients.
5. Economic viability of the targeting techniques.

Thus, the strategies of cancer and tumor targeting may prove to be an outstanding way of overcoming the adverse side effects of the traditional method of treating cancer and tumors.

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