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Strategies for targeted cancer therapy

Abstract

Numerous therapeutic measures have been developed in oncology to combat various types of cancer, which is a leading cause of death worldwide. These therapies range from conventional approaches to high-end precision medicines, all aimed at ensuring therapeutic efficacy and the patient's survival. However, drug resistance and off-target side effects continue to hinder treatment efficacy. In the case of solid tumors that have hypoxic regions that cause treatment resistance and a high risk of tumor recurrence. To address this, advanced therapies have been designed or under pipeline to specifically target cancer cells by considering their unique features. This review article primarily focuses on different treatment methods developed using the peculiar properties of the tumor microenvironment.

Keywords: tumor microenvironment, targeted therapy, hypoxia, nanoparticles, radiation therapy, chemotherapy, combination therapy, radiosensitizer, photodynamic therapy, immunotherapy, hyperthermia

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Introduction

Tumors are unusual masses of tissues characterized by undisciplined growth and proliferation of cells: these can be benign (non-cancerous) and malignant (cancerous). Cancer is the second-leading cause of mortality in human beings and refers to a group of diseases characterized by the development of malignant abnormal cells that divide uncontrollably and have the ability to spread throughout the body. Apart from uncontrolled proliferation, the other characteristics of cancer are evasion of apoptosis, angiogenesis, invasion of tissues and metastasis to different locations in the body. The proliferation and metastasis are the main causes of cancer mortality. Detection of cancer at an early stage increases the chances of complete cure of the disease. In spite of the human efforts to eradicate this disease from ancient times and the present day's spectacular achievements in science, technology and medicine, a definite cure for cancer is yet to be realized. There is a steady yearly increase in the number of new cases of cancer in both the developed and the developing countries.

Tumor - major treatment modalities

The available treatment modalities for cancer include – surgery, radiation therapy, chemotherapy, immune therapy, hormone therapy, gene therapy, stem cell therapy etc. Among these, surgery, radiation therapy, and chemotherapy are the most widely used.

Surgery: Surgery has been one of the major modalities of cancer treatment for many types of cancers especially when they are not spread to other parts of the body. It is also used to take biopsy samples for diagnosis and prediction of the stage of cancer. Combination of surgery with either chemotherapy or radiotherapy is often employed and found to be effective in curing cancer.

Radiation therapy: Radiation therapy or radiotherapy is the most important modality of cancer treatment. It is highly cost-effective and approximately 80% of all cancer patients require radiation therapy either for curative or palliative purposes.[1] The high-energy radiations deposit energy while passing through tissues causing ionizations to produce free radicals and damaging the vital biological targets - cellular DNA and membrane - resulting in the mortality of cancer cells.[2,3] Advances in imaging techniques, computerized treatment planning systems, radiation treatment machines (with improved X-ray production and treatment delivery), use of high-energy particles, etc. have contributed a great deal to the success of radiation therapy.[1] Due to rapid proliferation, the tumor cells overgrow their vascular supply, resulting in centrally necrotic and hypoxic regions where the cells are refractory to radiation. To overcome this problem, either cells of the tumor have to be sensitized to radiation by using hypoxic cell sensitizers or higher doses of radiation have to be used. Clinically use of higher doses of radiation is not possible as the normal cells, surrounding the tumor, are well perfused, vascularized and remain oxygenated, and therefore suffer more radiation damage. This necessitates the protection of the normal cells from radiation injury. Amifostin or ethyol is the only clinically approved compound available to protect normal cells.[4] Number of compounds (radiosensitizers) have been synthesized and reported to enhance the efficacy of radiation therapy.[5] Most of them completed preclinical studies successfully and failed in clinical trials due to toxicity to mammalian organisms. Also, several hypoxic cell sensitizers useful in radiotherapy of cancer are at different stages of clinical trials.[6] The nitrotriazole compound, Sanazole or AK-2123 (Figure 1), is an effective hypoxic cell radiosensitizer. The hypoxic cell radiosensitizing property of this compound and its lack of toxicity made it attractive as an adjuvant in radiation therapy of cancer. It has successfully completed phase III clinical trials and is used in clinics along with radiation to treat different types of cancers such as head and neck, cervical cancer etc. as a hypoxic radiosensitizer.[7]

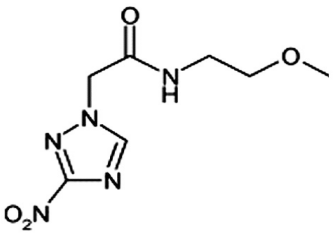

Figure 1 Chemical structure of Sanazole (Molecular Formula: C21H32N2O).

The treatment with Sanazole (SAN) enhanced radiation sensitivity in post-irradiated aerobic and anaerobic cells.[8] In patients with advanced head and neck cancer, the administration of SAN increased the sensitivity to hyper fractionated radiation treatment.[9] SAN has, also, the capability to enhance the therapeutic potential and reduce the effective dose of antineoplastic agents in comparison with the drug alone.[10–12] Konovalova et al.,[13] revealed that the therapeutic concentration of Sanazole demonstrated anti-metastatic activity in tumor-bearing mice. AK2123 has been found to augment the antineoplastic activity of the chemotherapeutic agent Mitomycin C

of multidrug resistant tumors.[13] According to Schepetkin et al.,[14] the bio-activation as well as the radiation and chemotherapy - sensitizing property of SAN could be due to the involvement of enzymes such as xanthine oxidase and microsomal NADPH/cytochrome p450 reductase.[14] The ability of SAN to accumulate in tumors was first demonstrated by Murugesan et al.,[15] via administering Technetium-99m labelled cyclam-sanazole to solid tumor-bearing animals. This study also revealed its potential in tumor imaging. Further, Das et al., explored the hypoxic tumor targeting capability of SAN.[16] The mechanism of sensitization of hypoxic tumor by SAN[17] is partially credited to its ability to induce increased DNA damage.[16] In human lymphoma cells (U937), SAN found to cause Fas ligand-induced Caspase 8 dependent apoptosis with the down regulation of hsp70 protein.[18]

Chemotherapy: Chemotherapy is a major therapeutic strategy in medical oncology using chemical agents or drugs to destroy cancer cells. Based on the mechanism of action, these agents are categorized mainly into alkylating agents, anti-metabolites, anti-microtubule agents, inhibitors of topoisomerase, and cytotoxic antibiotics.

Alkylating agents: These agents can alkylate macromolecules such as proteins and nucleic acids, basically derived from Mustard gas used in World War I.[19] They can damage genomic DNA, generate interstrand and intrastrand cross links in DNA resulting in inhibition of cell division (S-phase) and induce apoptosis. Other alkylating agents used in chemotherapy are cisplatin (Figure 2.1) and its derivatives (carboplatin and oxaliplatin), nitrosoureas, mitomycin, cyclophosphamide (Figure 2.2) etc.[19,20]

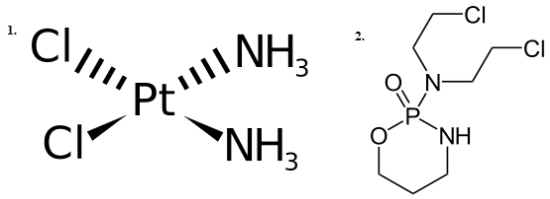

Figure 2 Alkylating agents. 1. Cisplatin and 2. Cyclophosphamid.

Anti-metabolites: They are usually the analogues of building blocks of DNA and RNA; hence interfere with the synthesis of nucleic acids. The cell cycle dependent activity of these agents induces the programmed cell death, apoptosis. Methotrexate (Figure 3.1) is an inhibitor of the enzyme, dihydrofolate reductase which decreases the synthesis of pyrimidine bases via inhibiting the production of folate coenzymes. Fluorouracil (Figure 3.2) is a nucleoside analogue which induces programmed cell death.[20,21]

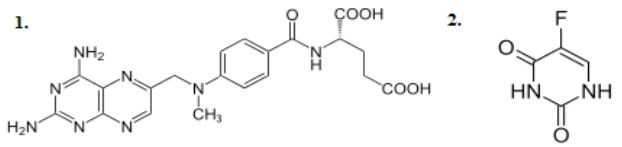

Figure 3 Anti-metabolites. 1. Methotrexate and 2. 5-fluorouracil.

Cytotoxic antiobiotics: Anthracyclines and bleomycins are under this group. Anthracyclines (eg. Doxorubicin; figure 4.1) are isolated from bacterium *Streptomyces peucetius.*

Doxorubicin: (DOX) is a well-known potent chemotherapeutic agent (Figure 4.1) approved by Food and Drug Administration, widely used against a range of cancers such as leukaemia, sarcoma etc.[22] DOX has the ability to fight with rapidly dividing cells, thereby decreases the tumor progression. The major mechanisms of action include DNA intercalation which prevents DNA replication and transcription, free radical generation, topoisomerase inhibition and apoptosis.[23] However, its therapeutic usage is limited only by its toxicity especially cardiotoxicity. DOX causes toxicity to cardiac tissues through the induction of oxidative stress,[24] reduced antioxidants,[25,26] altered heart-related expression of genes[27–29] and prevention of biomolecule synthesis.[30]

The antibiotic actinomycin can also intercalate DNAs and prevents the expression of genes.[31] Bleomycins (Figure 4.2) are obtained from *Streptomyces verticillus,* known to cause DNA intercalation; DNA strand breaks and free radical damage.[32]

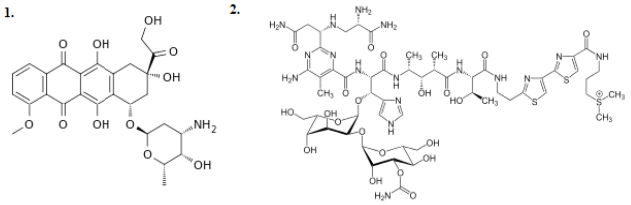

Figure 4 Cytotoxic antibiotics. 1. Doxorubicin and 2. Bleomycin.

Anti-microtubule agents and topoisomerase inhibitors: Most of these agents are plant-derived compounds. Several natural products have been tested as anticancer agents and some of them are in clinical trials. These products are usually the secondary metabolites of microorganisms and/or plants shows distinctive structural diversity which helps the organism to adapt to the various biological situations.[33,34] The major plants with clinically verified antineoplastic activity are *Catharanthus roseus*, *Camptothecin acuminata, Cephalotaxus harringtonia, Podophyllum peltatum*, *Taxus brevifolia, Viscum album, Annona bullata, Onchrosia elliptica, Rhizoma zedoariae* and *Asmina triloba.*[35]

The plant-derived anticancer agents in clinical use are categorized mainly into a) the vinca alkaloids, b) the epipodophyllotoxin lignans, c) the taxane diterpenoids, and d) the camptothecin quinolone alkaloid derivatives. Apart from these, there are several plant derived molecules which have anticancer activities and are useful in chemoprevention as well as treatment of cancer.

Vinca alkaloids: Vincaleukoblastine (Vincristine; Figure 5.1), an alkaloid isolated from *Vinca rosea* Linn shows anti-tumor activity in mice-bearing transplantable tumor.[36] Johnson et al (1960) reported the potential of anticancer agent vincaleukoblastine in tumor control via obstructing the essential cellular metabolic pathways.[37] According to Svoboda et al.,[38] leurocristine (Vinblastine; Figure 5.2), another Vinca alkaloid, has a wide anticancer activity in human tumors.[38,39] These alkaloids are known as 'spindle poisons' as they can interact with the cellular receptor, tubulin and prevents its assembly.[40,41]

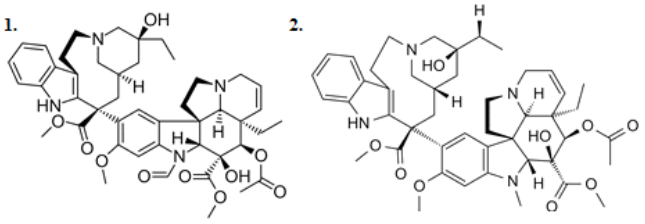

Figure 5 Vinca alkaloids. 1. Vincristine and 2. Vinblastin.

Epipodophyllotoxin lignans: The podophyllotoxin (Figure 6.1), a non-alkaloid lignan found to have antineoplatic activity, is isolated from *Phodophyllum* species.[42] It interacts with the enzyme topoisomerase II and prevents DNA unwinding as well as replication.[43]

Taxane diterpenoids: Taxol (Paclitaxel; Figure 6.2), isolated from *Taxus brevifolia*, is the first compound having taxane ring showed anti-leukemic and anti-tumor activities.[44] The mechanism of action of Paclitaxel is mainly through the interaction with 'tubulin', which prevents the disassembly of mitotic spindle and hence cell division.[45]

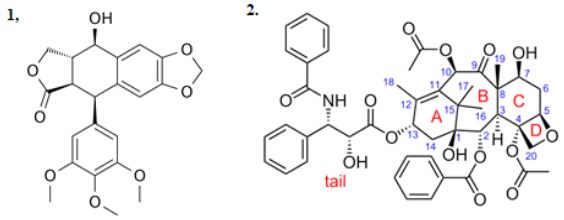

Figure 6 Structure of 1. Podophyllotoxin and 2. Paclitaxel.

Camptothecin quinolone alkaloid derivatives: Camptothecin (Figure 7), a quinolone alkaloid toxic to tumor cells, is extracted from *Camptotheca acuminata*. Topotecan and irinotecan, two analogues of Camptothecin clinically accepted as cancer chemotherapeutic agents, found to have anti-leukemic and inhibitory effect on tumors.[46] The mechanism of action is mainly through its interaction with the enzyme topoisomerase I.[47,48]

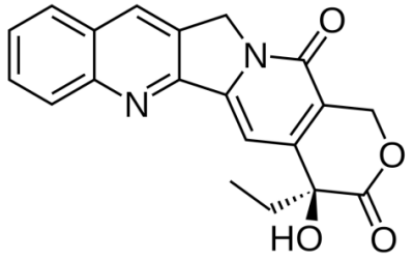

Figure 7 Structure of Camptothecin.

Curcumin: It is the most studied phenolic compound with anticancer property derived from roots of *Curcuma longa* L. Phase I and phase II clinical trials revealed the therapeutic potential of curcumin against various tumors.[49,50]

Flavopiridol: is a cytotoxic flavone in clinical trials found to induce cell death in human lung carcinoma cells[51] and also found to induce p53-independent programmed cell death in small cell lung carcinoma cells.[52] Lycopene is a tomato carotenoid shows a variety of biological functions, especially anticancer activities in various cancers, both *in vitro* and *in vivo* conditions.[53]

Resveratrol: a phenolic compound, found to interfere with tumor initiation and the various steps of tumor progression.[54] The prevention of growth of hepato-cellular cancer cells by resveratrol revealed its anticancer potential.[55]

Berberine: (BBN) (Figure 8) is an isoquinoline alkaloid derived from plants in Berberidaceae family such as *Berberis vulgaris* (barberry), *B. aristata* (tree turmeric), *B. aquifolium* (Oregon grape), *Hydrastis canadensis*, *Coptis chinensis* (golden thread) and *Arcangelisia flava* (Menispermaceae).[56] BBN is known to have anti-microbial, anti-helmintic, anti-viral and anti-inflammatory activities.[57–60] Recently, research has been focussing mainly on the antineoplastic activities of BBN. BBN might contribute anticancer activity through inhibiting the growth of *Helicobacter pylori*, an organism known to cause peptic ulcer and gastric cancer.[61,62] BBN also found to influence the activation of proto-oncogene to oncogene. The activation of proto-oncogene, c-Ki-ras2 contributes to oncogenic processes in human embryonic carcinoma cells - Tera 1 and Tera 2. BBN is found to influence the morphological differentiations of teratocarcinoma cells into neuronal cells through the negative regulation of c-Ki-ras2.[63] Since it was found that Activator Protein (AP-1) plays pivotal role in tumor progression.[64] BBN has an inhibitory effect on AP-1 activity as evidenced from a reporter gene assay done in human hepatoma cells.[65]

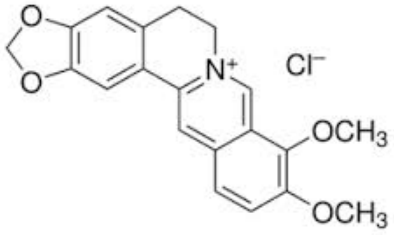

Figure 8 Structure of BBN.

Mantena et al (2006a and b) reported that BBN inhibits the growth of human epidermoid carcinoma A431 cells through cell cycle arrest at G1 phase and apoptosis via regulating Cdki-Cdk-cyclin cascade, activation of caspase 3 and poly (ADP-ribose) polymerase (PARP).[66,67] BBN was also reported to induce apoptosis in U937 cells while it enhances cell death in melanoma B16 cell line through the induction of apoptosis.[68]

Grisendi et al.,[69] reviewed the importance of Nucleophosmin/B23 in tumor progression as it was recognized as a potent tumor marker for several human tumors. The importance of the enzyme telomerase in cancer opens new avenues for developing strategies for cancer therapy.[70,71] BBN was shown to reduce the activity of telomerase and nucleophosmin/B23, and induce programmed cell death (apoptosis) in human leukemia HL-60 cells.[72] In 1969, Krey and Hahn[73] reported that BBN can interact with DNA molecules. Later in 1981 Rungsitiyakorn et al.[74] showed the influence pH in the binding of BBN to DNA. According to Liu et al (2008), the p53-associated cell cycle arrest, apoptosis and DNA double strand breaks were induced in human osteosarcoma cells by the treatment with BBN.[75] This BBN is found to induce cell death through apoptosis in human colon cancer cells by various biochemical reactions.[76]

Immunotherapy: Cancer cells express different molecules on its surface in order to enhance its proliferation. These molecules may be cancer antigens or carbohydrates. Immunotherapy is basically used to enhance the immune system by targeting these molecules on the surface, to kill cancer cells. The cell death mechanisms include antibody - dependent cell-mediated cytotoxicity (use antibodies to attack specific surface molecules), complement system (use blood proteins after antigen-antibody interaction), cell signalling (binding of antibodies initiates several signalling pathways to activate cell death mechanisms) and payload (antibody is conjugated with drug, toxin, small interfering RNA or radioisotope against antigen on the cell surface.[77–80] A combination of antibodies with radionuclides, radio-immunotherapy, has also been reported to be effective in cancer therapy.[81]

Photodynamic therapy: In this mode of treatment light-sensitive compounds, which are non-toxic to cells, have been used. When exposed to light these compounds become toxic. In the presence of light, photo sensitizers get excited and produce highly reactive free radicals, destroy the target cells. Some examples of photosensitizers are eg. aminolevulinic acid (natural), Allumera, Photofrin, Visudyne, Levulan etc. (commercially available). The application of photosensitizing agent to the body is purely depends on the part of the body being treated.[82]

Hyperthermia: The controlled use of high body temperature than normal is often capable to wipe out cancer cells. Exposing the cells to higher temperature than normal body temperature, cause alterations and make the cells more likely to be affected by the treatments such as radiotherapy and chemotherapy. Hyperthermia can be used in two different ways.

- Local hyperthermia or thermal ablation: Very high temperatures are used to kill a small area of cells.
- Regional hyperthermia or whole-body hyperthermia: A part of the body or whole body is exposed to slightly higher body temperature to destroy the cells and helps other cancer treatments work better. In whole body hyperthermia, body heated to 39°C to 43°C to treat cancer cells.[83]

Laser therapy: Laser, a narrow beam of light, has a single wavelength and can be used instead of scalpel in surgery. The commonly used types of lasers in these treatments are Carbon dioxide (CO_2), Argon, Nd:YAG (Neodymium: Yttrium-Aluminum-Garnet), Er:YAG (erbium: yttrium aluminum garnet); Ho:YAG (holium: yttrium aluminum garnet), copper vapor, and diode lasers [Lasers in Cancer Treatment. American Cancer Society].

- However, most of these treatment strategies are coupled with several harmful side effects and hence the therapeutic efficacy in terms of specificity gets compromised, causing damage to biological systems.[84] The treatments such as immunotherapy, laser therapy, hormone therapy and photodynamic therapy are still in the developmental stage. Most of the chemotherapeutic agents are known to cause systemic toxicities due to the lack of its therapeutic specificity. For example, the antineoplastic agents, Doxorubicin cause severe toxicity to heart tissues[85] and Cisplatin has been reported to generate renal injury in association with damages in tumor cells.[86]

The unique feature of all tumors is the rapid proliferation of the cells. The therapeutic strategy for tumor is particularly directed towards the rapidly proliferating cells. Chemotherapeutics predominantly affect rapidly proliferating tumor cells through interfering cell division, metabolic processes in the cells or cause damage to vital cellular targets such as DNA and membrane. However, rapidly dividing normal cells, such as cells of the bone marrow, intestine and hair follicles are also affected. The therapeutic effectiveness of drug increases with increasing doses of administration. However, with increase in doses of administration there is an increase in systemic toxicities and side effects which compromises the therapeutic dose.

By targeted delivery of drugs specifically to the tumor, one can achieve maximum therapeutic efficacy without side effects. Drugs can be delivered directly in the tumor by intratumoral injection at the site.[87,88] This is possible only in case of peripheral tumors. For most other tumors other means of delivering drugs have to be adopted. Intratumoral injection of carrier-based chemotherapeutics has also been tried.[89] Delivery vehicles such as liposomes[90] and membrane sacs of red blood cell[90] have been tried and the success was limited. Thermo labile liposomes carrying the therapeutics are of great advantage,[91] following administration, increasing the temperature at the tumor site can specifically release the contents in the tumor, while the liposomes remaining intact in other tissues. This would specifically destroy the tumor without affecting normal tissues.[92] The recent upsurge in nanotechnology and nano-medicine has contributed to the development of elegant novel strategies for delivering drugs to the tumor.[93,94]

Nanoparticles - the drug delivery vehicle

The nanoparticles or nanomaterials have distinctive optical, magnetic and electronic properties, and are capable to carry therapeutic or diagnostic agents. By utilizing these unique properties including their large surface-to-volume ratio, it is possible to develop new theranostic strategies. Since 1980s to the present, several technologies were implemented to enhance the activity and clinical success of nano-based therapeutics. The concept of PEGylation (poly ethylene glycol conjugation) was found to enhance the biopharmaceutical properties of proteins and biologically active substances.[95] The non-toxicity, water-solubility and less immunogenicity make PEG differ from other polymers.[96] 'Active targeting' of the drug can be achieved by conjugating the nanoparticles with specific ligand molecules for the cellular receptors, antibodies or peptides, providing specific interaction between receptors and ligands if they are in close proximity. Béduneau et al in 2007[97] demonstrated the effectiveness of active targeting by conjugating lipid nanocapsules of functionalized PEG with monoclonal antibodies against transferrin receptors (TFR) which are over expressed in cerebral epithelium, to facilitate specific drug delivery to the brain. Antibody-mediated cancer treatment was demonstrated by Daniels-Wells and Penichet (2016) using TFR-1 as a potent target.[98] Several PEGylated products are under various stages of clinical trials and some are in the clinic such as Doxil® (liposome-doxorubicin product) and albumin-based nano-drug carriers (Abraxane® - nanoparticle-albumin-paclitaxel and Albuferon-α® - albumin and interferon-α).[99] The conjugation of a new targeting peptide SP90 with doxorubicin-encapsulated liposomes was found to enhance the therapeutic index by improving its accumulation in tumors and reducing the drug-induced systemic toxicities.[100] For tumor mitochondria specific photodynamic therapy, Wei et al.,[101] developed a surface-modified Grapheme oxide based nano-drug in conjugation with the integrin αvβ3 monoclonal antibody.[101]

The passive targeting of nanoparticle-drug complexes is purely based on the phenomenon Enhanced permeability and Retention Effect. These nano-sized particles are entrapped by solid tumors because of their leaky blood vessels and inefficient lymphatic drainage system.[102,103] The size, shape, surface chemistry and stability[104] of nanoparticles have influence on its cellular uptake,[105] plasma clearance and bio-distribution.

The receptor-ligand interaction and subsequent downstream signalling cascade is influenced by the size of the particles. The gold and silver nanoparticles with size less than 100nm were effective to induce cell mortality.[106] The particles of size greater than 150nm will be cleared through reticuloendothelial system mediated by macrophage activation, while particle having size less than 10nm gets removed through renal clearance. Nanoparticles of the size 10-100nm will have good pharmacokinetic properties.

As the size of the particles increases above 150nm, the chance to get cleared from the circulation increases. The serum proteins get adsorbed on these particles coated with targeting molecules, prevents the accumulation of nano-complexes to solid tumors. The PEGylation of nanoparticles can improve the circulation time and passive targeting by inhibiting the interaction with serum proteins preventing macrophage - mediated plasma clearance.[107–109]

The enhanced retention can further be improved by coating with cell specific targeting molecules. Several studies reported that the size and surface chemistry including surface charge of nanoparticles have a strong impact on targeted delivery and circulation time of the particles.[110,111] The smaller particles, in comparison with larger particles, can also penetrate deeply in to the tumor interstitium.[112]

Magnetic nanoparticles in targeted drug delivery

Nanoparticles can be of non-metallic or metallic origin. Non-metallic NPs constitutes natural carbohydrate polymers like chitin, chitosan, carrageenan, polylactic acid etc. Metallic NPs comprise oxides as well as salts of several metals including silver and gold. Nanoparticles containing paramagnetic elements such as iron, manganese etc. will have magnetic property and are called as magnetic nanoparticles. Iron-oxide nanoparticles - Fe_3O_4 and gamma Fe_2O_3 - are of special relevance. These are used in large number of studies for diagnostic and therapeutic purpose. Our tissues and blood do contain iron and iron-oxide nanoparticles are biocompatible. These NPs are cost-effective compared to several other nanoparticles of metals.

Magnetic iron-oxide nanoparticles (NP) have gained attention in cancer diagnosis (Imaging) and therapy (drug delivery). Because of the magnetic property, they can be directed to specific areas in the body by the application of an external magnetic field. Super-paramagnetic NPs can be used as contrast agents in magnetic resonance imaging of tumors (MRI).[113,114] Several magnetic NPs are in the clinic as contrast agents such as AMI-25 for liver/spleen imaging, AMI-227 for lymph node imaging (size is 20-40nm), NC100150 (Clariscan, size is 20nm) for perfusion imaging and NC100150 for MR angiography.[115] NPs have been extensively employed as drug delivery vehicle in several studies. The importance of NPs in tumor therapy is mainly due to its capability to 1) transport and localize under the influence of an external magnetic field, 2) generate hyperthermia in the presence of alternating magnetic field and 3) carry targeting molecules to enhance active drug delivery.

Magnetic hyperthermia: IONP are capable of generating heat (hyperthermia) in the presence of an alternating magnetic field. The quantity of heat generated depends merely on the magnetic properties of the material and intensity of the magnetic field.[116,117] Under hyperthermia (40-46^{0}C) cancer cells cannot survive, while normal cells are unaffected.[118] The altered microenvironment makes the tumor cells more sensitive to higher temperatures with the exception of central nervous system (CNS).[119] As CNS was found to be sensitive at temperature 40-43°C for more than 6hrs, it is possible to treat tumor associated with CNS only under special conditions.[120] During hyperthermia (41.8°C), cytoplasmic heat shock protein 72 has been reported to over express on tumor cells, which is absent in normal cells.[121]

Magnetic drug targeting: The magnetic property has given more attention to magnetic NPs in solid tumor therapy as these particles can be attracted to a desired region with the application of magnetic field either external or internal. Hence, the non-specificity associated with conventional chemotherapy can be overcome by complexing them with magnetic NPs.[122] For the drug targeting, these nanocarriers should be biocompatible, hydrophilic and non-toxic. As mentioned earlier in this review; the diameter, shape, surface charge, surface modifications and composition of the magnetic nanoparticles have influence on magnetic property and drug delivery. Ma et al.,[123] developed a targeting strategy to overcome the deleterious effects of conventional chemotherapeutic agent, doxorubicin (having systemic toxicities, particularly cardiotoxicity and hepatotoxicity) by conjugating it with spherical carbon magnetic nanoparticles of size 40-50nm.[123] Doxorubicin was specifically targeted to solid tumor by conjugating with NPs and application of magnetic field externally[124] in an animal model. The clinical application of magnetic targeting of 4'-epidoxorubicin, with an external magnetic field, was successfully demonstrated in patients with solid tumors,[125] proving the advantage of this technique over conventional therapy.

The tumors which are >2cm away from the periphery of the body, cannot be targeted by the application of an external magnetic field because the strength of magnetic field decreases with increase in distance.[126] Takeda et al.,[127] demonstrated the essential application of magnetic NP - drug targeting to tumors, which are deep in site, using super conducting magnets.[127] Use of magnetic field internally to target NP-drug complexes to tumors was also investigated. The NP was coated with doxorubicin and this complex was targeted to tumor by implanting magnet inside the desired region using laparoscopic technique.[128] However, this strategy of drug targeting was found to cause several problems while applying to human situations.[129]

Magnetic nanoparticles in gene delivery: The magnetic NPs can also be used as a carrier for the delivery of nucleic acids to cells as nucleic acids can bind to magnetic IONPs.[130] This binding property has been exploited in the purification of DNA.[131] This gene transfer-mediated by the magnetic nanoparticle is called magnetofection. Thus, both RNA and DNA can be conveniently transport using this technique. The magnetic NP–DNA complex can be taken up by cells and the genes can be expressed in the recipient cells. This technique is extremely useful technique for controlling gene expression by introducing antisense oligonucleotides[132] and siRNA.[133] Nucleic acid can be conveniently attracted towards magnetic nanoparticle by coating them with polyethyleneimine having positive charge.[134] The nucleic acid complexed with NPs is taken up by the cells. Magnetofection can reduce time of transfection and the dose of the vector. The uptake of the complexes by the cells can be enhanced by the use of dynamic magnetic field through oscillating high intensity magnetic field. Recently, *in vivo* applications of the magnetofection with enhanced tissue penetration by oscillating magnetic field have been demonstrated.[135] The oscillating magnetic field imparts extra energy to the system which in turn results in particle uptake against external barriers. The underlying mechanism involves non-linear motion of the particles under the influence of the oscillating magnetic field facilitate tissue penetration, overcoming external barriers like muscle surrounding the tumor. Carbon nanotubes coated with nickel have also been found useful in transferring DNA to the cells under *in vitro* condition using magnetic field.[136] Oxidative therapy using complexes of magnetic NPs and D-aminoacid oxidase have been demonstrated in an animal model. This has proved even enzymes can be specifically targeted to tumor using magnetic NPs with the help of external magnetic field.[137,138]

Tumor microenvironment: challenges and opportunities

A new era in cancer therapy relies on precision medicine in which the treatment is tailored to the unique features of the tumor of an individual patient. This approach considers various factors such as genetics, lifestyle, and the unique features of cellular and molecular events of the tumor and its microenvironment. Here, tumor microenvironment plays a major role in customising therapy, and its inimitable features provide ample opportunities for designing diagnostic and therapeutic strategies. These features include an altered extracellular matrix, undeveloped leaky vasculature, hypoxia, acidity, lack of lymphatic drainage etc. which support cell proliferation and metastasis. This heterogeneity of tumor microenvironment together with genetic and epigenetic changes contributes to tumor progression and drug resistance.[139–141] Hence, a proper understanding of these interlinked characteristics of the tumor microenvironment, especially at its cellular and molecular levels is essential for better therapeutic gain. Figure 9 showcases the interconnected features of tumor microenvironment.

The (Figure 9.a) illustrates interlinked characteristic features of the tumor microenvironment. Figure 9.b depicts oxygen-dependent altered signalling pathway of Hypoxia-inducible factor-1α (HIF-1α): Under normoxic conditions, the enzyme with prolyl hydroxylase domain hydrolyses HIF-1α which promotes polyubiquitination by von Hippel-Lindau tumor suppressor protein (vHL). When the prolyl hydroxylase domain becomes inactive under a hypoxic environment and prevents hydroxylation, HIF-1α translocates to the nucleus, dimerizes with HIF-1β and recruits other factors. Ultimately, this promotes the binding of HIF-1α/ HIF-1β complexes to hypoxia-response elements (HRE) which leads to the activation of hypoxia-dependent downstream signalling pathways.

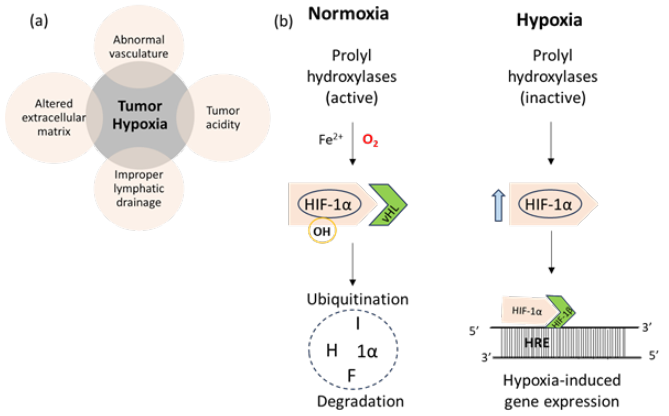

Figure 9 Showcases the interconnected features of tumor microenvironment.

Extracellular matrix (ECM): a dynamic contributor to tumor progression

The ECM is a complex network of highly cross-linked proteins that provide architectural support to the cells and control cellular activities in tissues. It is found in interstitial forms within organs and in specialized forms such as basement membranes and vascular endothelium, surrounding certain tissues and cell types. The ECM comprises proteoglycans and several fibrous proteins, mainly collagens- most abundant, elastin, fibronectins and laminins.[142] Changes in the organization of ECM are the hallmark of cancer progression, initiating a rewiring of cellular and molecular signalling that nourishes tumor progression. It includes altered integrin signalling and collagen degradation. Along with collagen deposition, the overproduction of lysyl oxidase contributes to ECM stiffness and the loss of its integrity, altering the downstream signalling cascade and promoting tumor progression.[143,144]

Heterogeneity in tumor vasculature is the basis for unique physiology

In normal tissue systems, the vasculature is hierarchically organized and regulated by the balanced expression of pro-angiogenic and anti-angiogenic factors based on metabolic demand. However, in tumors, the aggressive growth and proliferation of cells demand the hyper-activation of pro-angiogenic factors, leading to the improper development of vascular networks. The blood vessels in tumor tissues are heterogeneous and contain both normal blood vessels from which tumor cells invade and tumour-induced microvasculature. These blood vessels are often dilated and convoluted and have a branching pattern entirely different from normal vasculature. The altered endothelial linings, defective basement membranes and loss of pericytes make them leaky. Functionally, the immature blood vessels interrupt the proper supply of oxygen and nutrients to tumor cells, and the absence of functional lymphatic drainage hinders the clearance of waste materials from tumor mass.[145,146]

Tumor hypoxia and acidity

Tumor hypoxia is a medical condition where tissue oxygen levels are significantly lower than normal. This is caused by the rapid cell proliferation and growth of tumors, which restricts the invasion of blood vessels into the tumor. Cells far away from functional blood vessels are presumed necrotic, while cells in the hypoxic region are viable but have a lower proliferation rate and/or are in the resting G0 phase of the cell cycle.[147,148] Further nutrient deprivation in hypoxic cells can lead to the migration of hypoxic tumor cells into the necrotic zone. As the tumor progresses, this process continues and helps to maintain tumor survival. Most anticancer therapies are primarily effective against rapidly proliferating cells, which means that hypoxic cells often escape treatment.[149] The cells in the normoxic region that are dividing rapidly are highly exposed to treatments and undergo cell death, leading to improved nutrient supply to the previously hypoxic cells, allowing them to divide rapidly to regenerate the tumor. Oxygen availability plays a crucial role in the effectiveness of radiation therapy. Research has shown that radiation damages cancerous cells depending on oxygen availability during irradiation.[150–152] Therefore, hypoxic regions in solid tumors require higher doses of radiation for mortality, making them more resistant to treatment. Tumor cells follow an anaerobic glycolytic pathway for energy generation which contributes to the greater demand for nutrients for the uncontrolled rapid growth and proliferation of tumors.[153,154] Due to the absence of a proper lymphatic drainage system, the major by-products of this metabolic pathway accumulate in hypoxic tumor cells resulting in tumor acidity.[155]

Hypoxic environment in tumor: a feasible target for tumor therapy

Solid tumors have altered diffusion geometry that results in reduced oxygen and nutrient supply, leading to the formation of a hypoxic environment. The cells in the hypoxic zone, which are alive but in a quiescent phase (G0), promote malignancy and therapeutic resistance by activating genes responsible for tumor growth and metastasis.[156] Among them, hypoxia-inducible factor-1 (HIF-1) plays a crucial role in hypoxia-induced tumor progression and metastasis.[157] HIF-1 was identified by the recognition of the hypoxia response element (HRE) in the 3' enhancer region of the gene erythropoietin, a hormone involved in erythrocyte proliferation and undergoes hypoxia-induced transcription.[158,159] The heterodimer HIF-1 comprises a cytoplasmic subunit HIF-1α and a nuclear subunit HIF-1β. The oxygen-dependent regulation of HIF-1α is schematically presented in Figure 9.b.

The best mechanism behind the regulation of HIF-1α protein is mediated by the von Hippel-Lindau (vHL) protein. In the presence of oxygen, vHL protein recruits HIF-1α for ubiquitination via the 26S proteasome degradation pathway. Prolyl-4-hydroxylase (PHD) hydoxylates prolyl residues in HIF-1α, promoting the binding of HIF-1α with vHL. However, in hypoxic conditions, tricarboxylic acid cycle intermediates such as succinate and fumarate, or mitochondrial reactive oxygen species inhibit PHD activity and stabilize HIF-1α. The accumulated HIF-1α in association with the nuclear subunit HIF-1β binds to the HRE in target genes that promote hypoxia-responsible gene expression.[160,161]

The promoter region of vascular endothelial growth factor (VEGF), an important angiogenic factor - contains the HRE which is a binding site for HIF-1. This binding initiates VEGF expression which in turn promotes angiogenesis and subsequent tumor progression.[162]

In addition, the hypoxia in tumor milieu enhances the expression of epidermal growth factor receptors (EGFR). The EGFR signalling is known to enhance cell proliferation, prevent apoptosis and promote tumor angiogenesis and metastasis.[163,164]

Hypoxia targeted drug delivery

Selective therapy using hypoxia-activated pro-drug

There are two main types of drugs that target tumor hypoxia: quinone-based bio-reductive alkylating agents, such as mitomycin-C,[165] and nitroimidazole hypoxic cell radiosensitizers, such as Misonidazole.[166–168] In 1972, Lin et al. discovered that derivatives of benzoquinone can slow down the growth of adenocarcinoma 755 ascites cells and extend the lifespan of tumor-bearing mice. These compounds undergo alkylation after bio-reduction in hypoxic conditions.[169] In 1986, Zeman et al. proposed that the compound 3-amino-1,2,4-benzotriazine-1,4 dioxide (tirapazamine) can effectively kill cancer cells under hypoxic conditions as a selective bio-reductive agent.[170] Wilson pointed out the drawbacks of currently available hypoxia-selective cytotoxins as they are designed to destroy hypoxic cells which comprise a small fraction of the tumor population.[171] Therefore, it necessitates combination treatments with either radiation, chemotherapy or photodynamic therapy to eliminate the tumor population.

To address challenges, researchers created diffusible cytotoxins, such as nitro-deactivated aromatic mustards and cobalt (III) complex-deactivated aliphatic mustards, which have bio-reduction potential. These nitrogen mustards can cause DNA damage but with less cell cycle specificity. They become activated when reacting with reductive triggers like nitro and Co (III) in hypoxic cells. By exploring the use of hypoxia in its bio-reduction, most of the metabolic transformations of drugs were prevented in normoxic cells.[172] These drugs can become active through reductive metabolism in low-oxygen environments, producing toxic byproducts. The key mechanism behind this is that oxygen-sensitive enzymes, such as cytochrome p450 reductase, generate free radicals when reacting with these drugs. These free radicals can be toxic to cells, resulting in cell death.[173,174] However, when these drugs are metabolized by NAD(P)H dependent quinone oxidoreductase, which is not oxygen-sensitive, the two-electron product generated is less toxic in hypoxic cells than the one-electron product. In normoxic conditions, the free radicals generated through one-electron reduction react with oxygen to form superoxide radicals,[175] which are less toxic than the free radicals generated in hypoxic conditions.

Tirapazamine (TPZ) has been discovered to be cytotoxic in hypoxic conditions by Brown and Lee and Wilson.[173,176] The significance of TPZ (SR-4233) has been mainly attributed to its ability to enhance cytotoxicity in hypoxic mammalian cells[170] and improve toxicity to radiation. TPZ treatment, when combined with a well-known chemotherapeutic agent, Cisplatin, can enhance its anti-tumor potential in a hypoxia-dependent manner.[176,177] The therapeutic potential of TPZ has mainly been attributed to its protonated form in low-oxygen conditions.[173] However, recent evidence suggests that this protonated form is not the final toxic product. Rather, new radicals either hydroxyl or benzotriazinyl are involved.[178–180] Another pro-drug is Anthraquinone (AQ4N) which is activated to a hypoxia-selective cytotoxin through an unusual two-electron reduction mechanism achieved by the CYP3A members of the cytochrome P450 family.[181,182]

Hypoxia-selective gene therapy

Tumor-specific proteins that arise due to differential gene expression provide characteristic properties to tumor cells. This presents an opportunity for controlling tumors through gene-targeting methods. A new gene-directed enzyme prodrug therapy approach has been developed to deliver the genes of specific enzymes with promoters containing hypoxia-response elements. Once the gene transfer takes place, the genes are transcribed and translated to produce the required enzymes that convert a pro-drug to an active cytotoxic drug.[183] There are some clinical trials of this approach where the genes of enzymes that can activate prodrugs into cytotoxins are introduced into tumor cells. In 2003, Binley et al. developed an optimized hypoxia-responsive promoter in adenoviral vectors that stimulates the hypoxia-targeted expression of human cytochrome (CYP2B6).[184] This promoter interrupts tumor growth by activating prodrugs to active cytotoxic agents. Some of the most interesting enzyme/prodrug gene therapies are combinations of herpes simplex-1 virus thymidine kinase/ganciclovir and cytosine deaminase/5-fluorocytosine.[185]

Another approach in gene-directed enzyme pro-drug therapy involves transfecting a gene that encodes a one-electron reductase like cytochrome P450 3A4 for the hypoxia-specific activation of pro-drug AQ4N.[186] However, a significant challenge in this therapeutic approach is targeting the delivery of the enzyme/pro-drug to cells with high HIF-1 or hypoxia. An alternative strategy is to use a hypoxia-assisted adenoviral vector to transfect a reporter gene or a gene encoding the enzyme to human macrophages that can effectively activate the pro-drug as a cytotoxin.[187,188]

Interfering HIF-1 activity

Hypoxia-induced tumor growth can be prevented by interfering with the HIF-1 activity. Inhibiting HIF-1 can stop the major adaptive responses of tumor progression. Several approaches have been developed to regulate the potential of HIF-1. One method is inhibiting the HIF-1-associated transactivation of genes, like VEGF and epidermal growth factors, to prevent hypoxia-induced tumor growth. Kung et al. (2000) discovered that interrupting the interaction between HIF-1 and its co-activators p300/CREB with a polypeptide hindered the transcription of target genes involved in tumor progression.[189] Another approach is inhibiting the HIF-1 protein via translation or destabilization inhibition. Geldanamycin enhances the degradation of HIF-1 protein, which reduces its level in the tumor and leads to tumor regression.[190] The intratumoral introduction of an antisense HIF-1α-containing plasmid led to the downregulation of VEGF, which reduced microvessel density. Although this treatment did not show any effect on T cell-mediated immunity, it synergized B cell-, NK cell-, and CD8 T cell-dependent cure of tumors. The antisense HIF-1α gene therapy thus enhanced the efficacy of immunotherapy[191] and improved the therapeutic efficacy of doxorubicin.[192] There have been significant advancements in the development and application of HIF-1α inhibitors for controlling tumors. Studies have shown that the chemotherapeutic drug cisplatin can enhance HIF-1α degradation in ovarian cancers that are sensitive to cisplatin.[193] An antisense oligonucleotide, identified as EZN-2698, has successfully completed a phase I clinical trial in patients with advanced solid tumors.[194,195] Also, a HIF-1 inhibitor called topotecan has completed clinical trials in non-small cell lung cancer.[195] Furthermore, the anti-tumor activity of gemcitabine has been demonstrated to induce immunogenic cancer cell death in pancreatic ductal adenocarcinoma by inhibiting HIF-1α through PX-478.[196]

Therapy with anaerobic bacteria

Anaerobic bacterial therapy is relevant due to the necrotic regions in tumors where blood supply and oxygen are absent. The use of bacterial therapy has been documented for over a hundred years,[197] and active research is still being done in this area. Recent

developments in this strategy involve targeting therapeutic agents to anaerobic necrotic areas in the tumor using a genetically engineered non-pathogenic strain of the bacterial genus Clostridium that can grow and localize in these regions.[198,199] The major approaches in bacterial therapy include using bacteria to enhance the therapeutic potential of drugs, as carriers of anti-neoplastic drugs, and as vectors in gene therapy.[200] However, the most favorable approaches are the use of genetically modified bacterial strains for tumor destruction and bacterial gene-directed enzyme/pro-drug therapy. The gene encoding cytosine deaminase, an enzyme present in *Escherichia coli* that can convert pro-drug 5-fluorocytosine to the cytotoxic chemotherapeutic agent 5-fluorouracil, was cloned in Clostridium using an expression vector. This method could increase the sensitivity of murine EMT6 carcinoma cells to 5-fluorocytosine several-fold.[201] Gardlik et al. reviewed the approach of Bacteria-mediated anti-angiogenesis therapy in tumor tissues.[202]

Combination therapy and chemotargeting

Use of more than one therapeutic agents or drugs has shown to be great advantage in many instances as combination of drugs with different mechanisms of action provide synergism in cancer therapy which could also prevent the treatment associated multi-drug resistance. Highly potent combinations of drugs are quite often associated with deleterious effects due to toxicities and side effects. Nanoparticle combinations of drugs are an alternative to overcome these deleterious effects.[203] Single nanoparticle combinations of multiple drugs have great advantage over combined administration nanoparticle – single drug combinations since the former offers uniformity of the vehicle size, proper loading of drugs in desired proportion and time-dependent time release.

Nanoparticle platforms such as liposomes, dendrimers, polymeric nanoparticles etc. are employed in many instances for the co-delivery of chemotherapeutic drugs, siRNA, sensitizers etc. for tumor control.[204,205] Specific targeting of neoplastic drug doxorubicin to tumors have been achieved by complexing them with nanoparticles of iron-oxide and silver-oxide together with a hypoxic sensitizer Sanazole.[206,207] Targeting of the tumor by external magnetic field is effective in case of peripheral accessible tumors but this method is not suitable for deep seated internal tumors and for these chemotargeting could be effective. Chemotargeting of cytotoxic drugs to tumor could be achieved by utilizing the peculiar properties of tumour microenvironment, particularly the hypoxia in malignant tumors. Following administration, aromatic nitro compounds such as nitroimidazoles and nitrotrazoles get accummulated tumour bearing animals get accumulated in the hypoxic tumor regions in tumor bearing animals. This property of the nitrocompounds to get accumulated in tumor microenvironment is best utilized for chemo-targeting of therapeutics to the tumor tissiue. In tumour bearing mice, oral administration of a complex containing chemotherapeutic doxorubicin (DOX) or the cytotoxic phytoceutical, berberine (BBN) along with iron oxide nanoparticles (NP) and the nitrotriazole compound, sanazole (SAN) result in accumulation of the nanocomplexes (NP-DOX-SAN) and (NP-BBN-SAN) specifically in the tumor site resulting in reduction of tumor volumes in tumor bearing mice.[206–208] Thus nanoparticles can be effectively used for chemo-targeting cytotoxic drugs to cancer cells and achieve better therapeutic outcome. However, more studies are needed to initiate clinical trials.

Future perspectives

The tumor specific proteins arising from differential expression of genes confer characteristic properties to tumor cells and provide opportunity for developing strategies of tumor control through gene targeting methods. Tumor cells in general display reduced apoptosis and if one can enhance the apoptosis, either by reducing the expression of anti-apoptotic proteins or increasing the expression of pro-apoptotic proteins, will have therapeutic benefit. Tumor hypoxia and associated expression of genes enhances tumor progression. Developing siRNA or antisense RNA techniques for the inhibition of expression of these genes (*vegf*, *egfr*, etc.) can be thought of as therapeutic strategy for tumor control. Certain killer peptides/proteins are expressed in cells which are in severe stress and trauma.[209] These peptides trigger pathways to induce cell death. Tumor can be controlled by specific targeting of killer peptides to them using any of the nanocarriers. Thus, nanotechnology and the characteristics of tumor microenvironment can be harnessed to develop suitable strategies for tumor control.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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