



REVIEW ARTICLE

CNTs mediated CD44 targeting; a paradigm shift in drug delivery for breast cancer

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Abstract The breast cancer is one of the most common cancer affecting millions of lives worldwide. Though the prevalence of breast cancer is worldwide; however, the developing nations are having a comparatively higher percentage of breast cancer cases and associated complications. The molecular etiology behind breast cancer is complex and involves several regulatory molecules and their downstream signaling. Studies have demonstrated that the CD44 remains one of the major molecule associated not only in breast cancer but also several other kinds of tumors. The complex structure and functioning of CD44 posed a challenge to develop and deliver precise anti-cancerous drugs against targeted tissue. There are more than 20 isoforms of CD44 reported till date associated with several kinds of tumor in the using breast cancer. The success of any anti-cancerous therapy largely depends on the precise drug delivery system, and in modern days nanotechnology-based drug delivery vehicles are the first choice not only for cancer but several other chronic diseases as well. The Carbon nanotubes (CNTs) have shown tremendous scope in delivering the drug by targeting a particular receptor and molecules. Functionalized CNTs including both SWCNTs and MWCNTs are a pioneer in drug delivery with higher efficacy. The present work emphasized mainly on the potential of CNTs including both SWCNTs and MWCNTs in drug delivery for anti-cancerous therapy. The review provides a comprehensive overview of the development of various CNTs and their validation for effective drug delivery. The work focus on drug delivery approaches for breast cancer, precisely targeting CD44 molecule.

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Abbreviations: SWCNTs, Single-walled Carbon Nanotubes; MWCNTs, Multiwalled Carbon Nanotubes; CD 44, Cluster of Differentiation; HA, hyaluronic acid; BBB, Blood–Brain Barrier; siRNA, Small Interfering RNA; DNA, Deoxyribonucleic acid; MMPs, Matrix metalloproteinase; HNSCC, Head and neck squamous cell carcinoma; PTT, Photothermal Therapy; PDT, Photodynamic Therapy.

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CD 44 and role in physiology

CD44 a transmembrane glycoprotein associated with several vital functions, including cell division, migration, adhesion, and signaling.¹ Over the last few decades, the role of CD44 has been extensively studied in context with cancer not only breast but much other cancer as well. In several research findings have demonstrated altered expression of CD44 and malfunction during inflammation response and cellular damage.² A preponderance of research data demonstrated overexpression of CD44 in many diseases, including cancer, autoimmune disorders, and inflammatory diseases.^{3–6} CD44 antigen present on the cell surface plays a vital role in cell–cell interaction and downstream signaling. Additionally, CD44 also involved in cell adhesion and migration. CD44 protein encoded by the CD44 gene located on chromosome 11 in human. CD44 gene encodes CD44 antigen among various mammalian cells and exist as several isoforms. Existence of various isoforms of CD44 antigen is the result of splicing events during maturation of mRNA.⁷ CD44 participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing, hematopoiesis, and tumor metastasis. Credit goes to Oxley and Sackstein, 1994 in the understanding the biology of CD44 interaction.⁸ CD44 is a receptor for hyaluronic acid (HA) and can also interact with other ligands as well such as osteopontin, collagens, and matrix metalloproteinase (MMPs).⁹ Additionally, post-translational modifications control CD44 functions and suggested post-translational modifications facilitate the transition of CD44 isoforms.

As a cell surface adhesions molecules, CD44 proteins associated in cell–cell and cell–matrix interactions expressed ubiquitously.¹⁰ Various isoforms of CD44 encoded by a single gene as a result of splicing mechanism precisely alternate splicing. Additionally, the multiple isoforms also result in several other post-translational modification events.¹¹ The role of CD44 in various diseases and its association in physiological functions studied in last few decades.^{12–15} The complex structural insights and interaction with native ligand hyaluronic acid studied for the unique association between ligand–receptor complexes.¹⁶ CD44 is highly promiscuous and interact with several other ligands including osteopontin (OPN), chondroitin, collagen, fibronectin, and serglycin/sulfated proteoglycan. Involvement of CD44 in various physiological events is largely due to its affinity with multiple ligands addition to native one, i.e. HA.^{17–21} However, preponderance research studies and scientific data demonstrate that HA most specific and robust ligand for CD44. Further, all the CD44 isoforms have shown an affinity for HA in altering magnitude.²² Apart from HA, other ligands have limited affinity for CD44 shows association in limited cellular functions. Expression of CD44 and outrage of various isoforms as a result of alternative splicing and post-translational modifications actively regulate various cellular signaling molecules including Hippo signaling, β -catenin, TGF- β , Emmprin (CD147), Matrix metalloproteinases and STAT3.²³

In human CD44 gene is composed of 19 exons encoding several isoforms of CD44 as a result of alternative splicing. The standard CD44 (85–95 kDa) encoded by first five and

last five exons.²⁴ The role of CD44v3, CD44v5, and CD44v6 isoforms in breast cancer for metastasis was studied.²⁵ Further, few other CD44 isoforms including CD44v5, CD44v6, and CD44v7-8 reported linked with lymph node metastasis.²⁶ The role of CD44 among various metabolic pathways remain complex however, standard CD44 and CD44s trigger Wnt/ β -catenin signaling pathways. CD44 and inflammatory response are closely associated.²⁷ On the contrary, several cytokines including TNF α and IFN γ modulated CD44-9v and CD44-6v expression. Additionally, cytokines, including IL-1, IL-4, GM-CSF, or TGF- β have a minimal effect of CD44 expression (CD44-9v and CD44-6v).²⁸ The study also demonstrated that CD44s activates TGF- β signaling pathway associated with several cellular functions, including the control of cell growth, cell proliferation, cell differentiation, and apoptosis.²⁹ Anti-apoptotic event is essential to control tumor development, and CD44/CD44v6 reported promoter of anti-apoptotic proteins.³⁰ The role of CD44 in energy harvesting metabolic pathways was studied and reported it controls glucose metabolism in various tumor cells including prostatic SCNC. The study also demonstrates CD44 alters glycolytic enzymatic activity and providing ideal environment for tumor cells.³¹ However, there is limited study demonstrating precise role of CD44 isoforms in human physiology and cancer progression.

CD44 and cancer

CD44 role in the development of various kinds of tumor and cancer has been studied extensively during the last few decades.³² Studies have demonstrated that CD44 involvement in several metabolic pathways and physiological events associated with tumor development.³³ CD44 is a multi-structural and multifunctional cell surface receptor molecule associated in cellular events including cell proliferation, cell differentiation, cell migration, angiogenesis, and presentation of inflammatory mediators such as cytokines, chemokines, and growth factors to the corresponding receptors.³⁴ CD44 also involved in the recruiting of proteases at the cell membrane, as well as in signaling for cell survival.³⁵ Under normal circumstances, these physiological properties regulate normal cellular development and maintain homeostasis.³⁶ On the contrary, the CD44 fail to deliver the mentioned function in tumor and a cancerous cell. Quere et al, 2011 have studied overexpression of CD44 gene result in high levels of the adhesion molecule CD44 resulting leukemia.³⁷ As mention earlier, CD44 possess an affinity for several ligands and mediated carcinogenesis in a differential manner. Fujita et al, 2002 studies the molecular mechanism behind CD44 mediated carcinogenesis.³⁸ The HA is a complex signaling cascade where the affinity of HA with the CD44 result in tyrosine phosphorylation and activation of FAK linked with PI3K. The study also suggested that cells expressing CD44 develop resistant against induced apoptosis precisely via etoposide.³⁹

Brown and colleagues demonstrated that a higher chance of isoforms transition of CD44 in tumor cells develops various kinds of tumor and cancers.⁴⁰ Another study demonstrates an additional peptide as a result of CD44

isoforms mechanism at the juxtamembrane domain involved in signaling conformational modulation by providing affinity surface for inflammatory molecules, including cytokines and growth factors.²³ The prevalence of cancer in pancreatic tissues is a classic example of overexpression of CD44 and its isoforms. CD44v6, a dominant variant of CD44 in pancreatic cancer tissues, reported.⁴¹ CD44 overexpression and isoforms frequency studied for its role in metastasis as well in pancreatic cancer, tissues.⁴² The expression of CD44 and its variants do not alter during treatment with inflammatory molecules and growth factors like bFGF, EGF, TNF α , and TGF- β 1. The overexpression of CD44 was reported in prostate cancer as well with 42% of PCa, 57% of HGPIN, and 42% of BPH tissues.⁴³ Moura et al, 2015 studied the expression profile of standard and variants forms of CD44 related to prostate cancer behavior.⁴⁴

In a subsequent study, Hernandez et al, 2015 confirmed the altered expression profile of CD44 isoforms in prostate cancer using flow cytometry and Western blot analysis.⁴⁵ The small interfering RNA molecules (siRNA) were implemented in studying the functional role of CD44 isoforms variant CD44v6 in prostate cancer cells Ni et al, 2014. Here, in a knock-down study of CD44 variant CD44v6 result in loss of EMT markers and significant reduced tumorigenic potential, tumorsphere formation, and enhanced chemo/radio sensitivity.⁴⁶ The role of microRNA as a negative regulator for CD44 expression was studied. Down-regulation of several microRNA including miR-34a, miR-106a, miR-141, and let-7b in stem/progenitor cells which expressed CD44.⁴⁷ Further, a few microRNAs, including miR-301 and miR-452 were reported overexpressed in CD44-positive cells. In the head and neck squamous cell carcinoma (HNSCC) cell lines another variant of CD44, i.e. CD44v3 was evaluated and reported higher level than normal expression values.⁴⁸ The broad substrate affinity of CD44 and its expression subsequently was studied in tumor cell proliferation. Delta Np-63 directly regulates CD44 expression potentiated EGFR activation and the expression of ABCB1 multidrug transporter gene which contributed to tumor cell proliferation and chemoresistance in HNSCC.⁴⁹ A detailed mechanism for CD44 association with cancer development proposed in Fig. 1.

Research studies demonstrate that altered/abnormal expression of CD44 isoforms largely CD44v6 and CD44v10 associated in onset and progression of colorectal adenocarcinoma.⁵⁰ The CD44 variant CD44v6 shown increased expression in the presence of inflammatory cytokines HGF, OPN, and stromal-derived factor 1 α (SDF-1) in CSE and activated the Wnt/ β -catenin pathway indulge in migration and metastasis.⁵¹ Additionally, a few CD44 variants were reported altered functional role in the growth of tumor.⁵² The finding support concept that each of CD44 variant involves in tumor progression. This is mainly due to the promiscuous nature of CD44 develops an affinity for various ligands and hence deliver distinct function. However, the expression pattern and extent both play a vital role in drug resistance. In a study, it was reported that cells are having overexpression of CD44v6 treated with standard chemotherapeutic drugs shown significant higher viability and clonogenicity in colon cancer over cells which lacks expression of CD44v6 variants.⁵³

CD44 represents a class of cell surface adhesion glycoprotein plays a vital role in cell–cell interaction.

Role of CD44 in cancer development is well studied precisely metastatic determinant.²³ Further, the role of CD44 in breast cancer involves several factors, including CD44 expression pattern, CD44 splicing events, HA binding affinity, CD44 isoforms, DNA methylation events, and metalloproteinase. HA as a native ligand for most of isoforms of CD44 alters cell signaling that enhances cell adhesion, migration, and proliferation. Bourguignon et al, 2009 demonstrated that HA binding with CD44 results in expression of p glycoprotein (multidrug-resistant gene) along with Bcl (anti-apoptotic gene) in breast tumor cell. The study further concludes the expression of p glycoprotein and Bcl results in proliferation and survival of tumor cell.⁵⁴ Another study shows that in breast tumor cell expression of serglycin and CD44 core proteins increase by several folds promote cancer progression.⁵⁵ Expression of CD44 is highly dynamic and reported a transition between its isoforms CD44v to CD44s during alternative splicing promote breast cancer development.¹⁰ Nam et al, 2016 reported CD44 silencing significantly reduces consumption of glucose, ATP production and lactate production by cancer cell.⁵⁶ Further, β -catenin and AKT pathways are associated with CD44 expression. The expression of CD44 can be regulated by inhibiting β -catenin and AKT pathways alone and or in combination.⁵⁷ Nuclear events such DNA Methylation alters CD44 gene, and its expression studied in breast tumor cells. Further, metalloproteinase-2 (MMP-2) and MMP-9 expression level and breast cancer prognosis were studied. In a finding the mRNA levels of metalloproteinase-2 (MMP-2) and MMP-9 were reported significantly higher in CD44 positive breast cancer cells over CD44-negative cells.⁵⁸

Role of CD44 in breast cancer

Breast cancer is one of the most common cancers affecting women worldwide. The abnormal expression and functioning of the CD44 gene along with CD44 protein-mediated signaling result subsequently in abnormal growth of parenchyma cell in breast tissue.⁵⁹ The affinity of CD44, along with its several isoforms (variants) plays a crucial role in metastatic cascade via a high degree of cell surface receptor interactions.⁶⁰ The extracellular domain of CD44 can bind co-receptors, initiating recruitment, and activation of signaling cascades. Significant evidence supports its interaction with and influence on the ErbB family of receptor tyrosine kinases. Epidermal growth factor receptor (EGFR)/ErbB1 and ErbB2/Her 2 are key regulators of metastatic disease, and their expression is associated with the most aggressive forms of breast cancer.⁶¹ The binding of native, as well as a subsidiary ligand with the CD44 result in activation of Ras and SOS, mediated growth and invasion cascade. LMW hyaluronan precisely triggers an interaction with Grb2 and p185Her2, and it promotes CD44 binding to N-Wasp.⁶² The role of CD44 in Smad dependent invasion was studied and proposed that its TGF β receptor 1 and 2 trigger ankyrin–CD44 interaction. CD44 reported as an activator for enhancing tumorigenic signals mediated by a series of signaling events including activation of Rho GTPases, which promote cytoskeletal remodeling and invasion, and the PI3K/AKT and MAPK-Ras pathways, which promote growth,

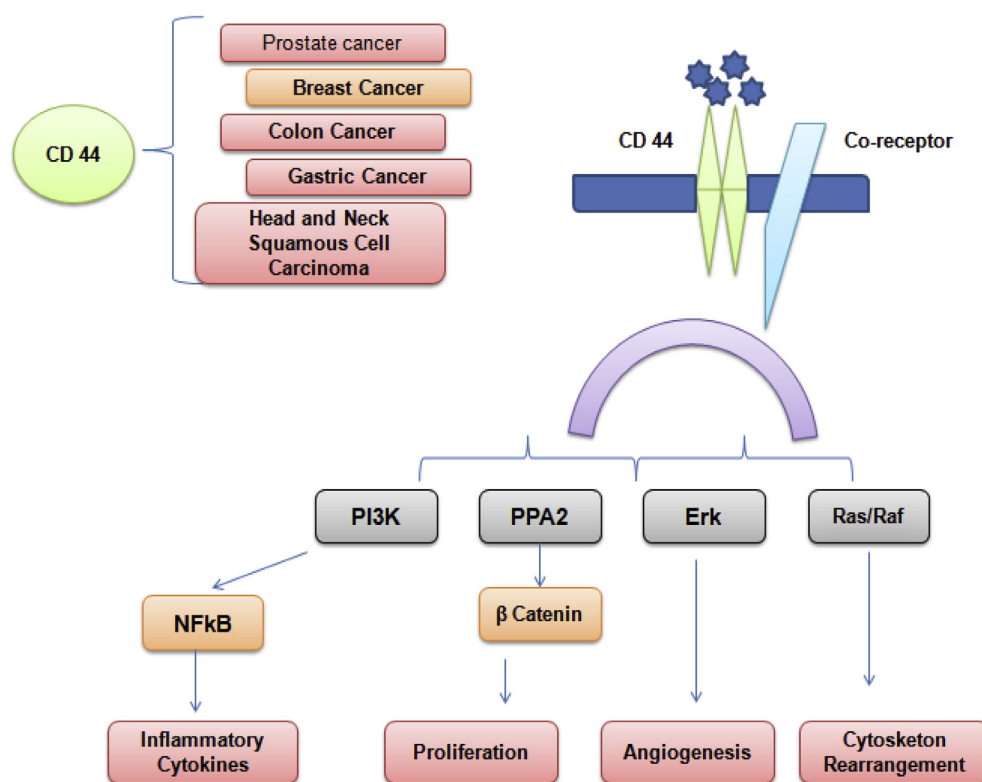


Figure 1 CD44 and its association in cancer development including breast cancer. A schematic depiction of various players and downstream signaling associated with CD44 and ligand interactions regulate several metabolic pathways including NFκB and β catenin.

survival, and invasion.⁶³ A tetracycline-inducible CD44 complex was studied breast cancer cell line and reported an enhancing tumorigenic outcome.⁶⁴ The role of CD44 in tumorigenesis was further studied using xenograft tumor model. Here, in this study, using a CD44-blocking monoclonal antibody had shown significant inhibition of tumor growth along with lack of recurrence in tumor cell treated with doxorubicin/cyclophosphamide.⁶⁵

Current approaches to drug delivery

Though several therapeutic and drug delivery system for anti-cancerous drug delivery targeting breast cancer are available, but most of these approaches possess own limitations and benefits.⁶⁶ The conventional methods, including chemotherapy, radiotherapy, and surgery are still in clinical practice as limited choices for advanced methods. There is a transition towards design in drug delivery methods for cancer therapy including breast cancer. The nanotechnology-based drug delivery vehicles have gained tremendous success in recent time.⁶⁷ Throughout decades of research, involved in drug delivery system for breast cancer focus in the development of micelle, liposome, polymers, and dendrimers for a large volume of anti-cancerous drugs.⁶⁸ However, gold nanoparticles, SPIO nanoparticles, and quantum dots are effective for a small dose of drug molecules.^{69,70} The major challenge in existing approaches for drug delivery for breast cancer is to develop a therapeutic specific to tissue. Subsequently, most of these methods are non-specific in drug delivery until Functionalized.

In a study, Dave et al, 2014 discussed the novel approach, i.e., transpupillary drug delivery to the breast to overcome breast cancer.⁷¹ Ahmed and Douek, 2013 demonstrated an increasing potential of magnetic nanoparticles in drug delivery for breast cancer.⁷² In their finding, they have reported magnetic nano-particles can offer much precise drug delivery to targeted tissue in a controlled release manner. Similarly, Li et al demonstrated the potential of transferrin-conjugated gold nanoparticles not only in anti-cancerous therapy but also cancer cell imaging.⁷³ Dhankhar et al, 2010 have reviewed the pros and cons of existing drug delivery system being used for breast cancer management.⁷⁴ In a recent study, Sadat et al, 2015 demonstrated the potential of a nano-formulation in selective drug delivery for breast cancer.⁷⁵ Here they have emphasized the specific recognition of HER2 receptors of HER2 overexpressed tumor cells, and evaluate anti-HER2 monoclonal antibody as an effective tool for active targeting.⁷⁶ Considering all these research findings, despite several novelties and robust approaches in anti-cancerous drug delivery including nanotechnology-based still, a cure for breast cancer seems incomplete which further suggest the need for an alternate in drug delivery vehicles.

Paradigm shift in drug delivery vehicles

With the enormous limitations of existing drug delivery approaches for cancer management, anti-cancerous therapy, there is an immediate need for a paradigm shift in hunting novel methodology.⁷⁷ The conventional therapeutics also

associated several limitations such as the inability of drugs lack tumor sites specifically, which brings difficulty in the clinical use of anti-cancerous drugs. Certainly, nanotechnology had a wide spectrum in nano-designs which can be an ideal cargo for anti-cancerous drug delivery.⁷⁸ The carbon nanotubes become an important and integral part of modern healthcare as effective drug delivery vehicles. The functionalized CNTs seem much effective and precise drug delivery.⁷⁹ The CNTs become an integral part as therapeutics in biomedical fields due to their unique structures and properties, including high aspect ratios, large surface areas, rich surface chemical functionalities, and size stability on the nanoscale.⁸⁰ The CNTs are being used as nanocarriers for anticancer drugs including doxorubicin, camptothecin, carboplatin, cisplatin, paclitaxel, Pt (II), and Pt (IV), and genes including plasmid DNA, small interfering RNA, oligonucleotides, and RNA/DNA aptamers.⁸¹ One major limitation associated with CNTs is cytotoxicity can be easily optimized using appropriate functional group using fictionalization. The advantage of using CNTs as a nanocarrier for anti-cancerous drug delivery is a wide range of cargoes including chemotherapeutic agents, nucleic acids, and protein molecules.⁸² Due to the advances in synthetic chemistry over the last few years, different biological nanomaterials developed, which can be used for a variety of biological therapies, such as drug delivery, cancer diagnosis, treatment, and imaging.⁸³ The interdisciplinary approaches by combining synthetic chemistry with biological engineering will surely open new avenue in therapeutic and other biomedical applications.

Drug targeting to breast cancer using nano-formulations, including CNTs and other nano designs mainly focus Hyaluronan-Based and folic acid-based targeted Delivery. CD44 effectively binds with hyaluronan or hyaluronic acid and stimulate downstream signaling cascade. Over-expression of CD44 reported in many malignancies, including breast cancer. Several studies have demonstrated that nano designs (Carbon Nanoparticles) effectively target and down-regulate expression of CD44 precisely in conjugated with HA. Folic acid is dietary entity and essential for synthesis of purine and pyrimidine building block of nucleic acid.^{84,85} In the case of breast cancer, along with CD44, folate receptor over-expression is reported. Studies have shown that folic acid-based targeted delivery effectively delivers drug to tumor cells. Aromatic ring with carboxylic moiety develops affinity for nano designs including functionalized CNTs.⁸⁶ These functionalized nano designs are effective carrier for drug delivery to various tumors including breast cancer.⁸⁷ Additionally, folic acid-functionalized MWCNTs serve as ideal target for several cutting edges imaging techniques magnetic resonance imaging (MRI), confocal fluorescence microscopy and confocal Raman microscopy.^{88,89}

CNTs and applications

Carbon Nanotubes (CNTs) have vast applications almost in every field, including biomedical, environment studies, biosensor, water purification, and agriculture. Over the last few decades, there is a tremendous advancement in biomedical applications of CNTs, including drug delivery system, tissue engineering, and grafting, tissue, and organ

implants.⁹⁰ The functionalized CNTs are an ideal choice for drug delivery not only for antineoplastic drugs but also several other drugs intended for vital tissues. The unique and highly symmetrical arrangements of CNTs provide an ideal platform for tissue engineering by providing a scaffold for growth of tissue and organ.⁹¹ The CNTs are promising drug delivery vehicles in delivering the drug to the neuronal tissue to combat several life-threatening diseases including brain tumor, cerebral thrombotic disorders, and infections.⁹² Several studies have demonstrated the potential of CNTs in delivering drugs including antineoplastic drug and nucleotide, e.g., siRNA and microRNA. Further CNTs were reported effective in treating several infectious diseases along with their diagnostic potential.⁹³ The use of CNTs in cancer management has gained tremendous success not only for drug delivery but also the early diagnosis. SWCNTs and MWCNTs both used in cancer management for a variety of cancer including breast cancer.⁹⁴ Antitumor chemotherapy requires administration of several drugs in various doses, and hence CNTs are ideal vehicles to tag targeted drugs for effective antineoplastic therapy. Here, Fig. 2 demonstrates a comprehensive overview of anti-cancerous drug delivery using CNTs.

The CNTs are unique and effective in drug delivery system due to their physicochemical properties and functionalized group. These physicochemical properties enable CNTs for a deeper penetration often require for solid tumor and drug-resistant tumor as well.⁹⁵ Further CNTs provide a wide range of drug tagging/loading enable a combination therapy for cancer management and other diseases as well. The studies have shown that CNTs with host-specific molecules can elicit immune response quicker and effectively by raising antibodies against targeted molecules.⁹⁶ Pristine SWCNTs exhibited an antimicrobial effect for a variety of infectious agents. These tiny structures are key for modern biosensor designs. CNTs provide a large surface to enable immobilization of detection molecules. The unique optical and thermodynamic properties of CNTs are the basis of their applications for diagnostic development.⁹⁷ Further, the intrinsic physicochemical properties of CNTs, including Raman scattering and photoluminescence enable tracking, detecting and imaging diseases in real-time. These potential provide the status of therapy in real-time along with the efficacy of the drug delivery system. Poor dispersibility is a major challenge using CNTs in biomedical applications result in a small volume of distribution (Vd) (a). The lower Vd enhances dose-dependent toxicity and biocompatibility of CNTs associated drugs. CNTs are often associated with tissue-specific toxicity, and hence functionalization with appropriate group reduces toxicity to the significant level.⁹⁸

Applications of SWCNTs

Chemotherapeutics are major anti-cancerous drug intended for the treatment of various forms of cancer alone and along with other drugs and approaches, including chemotherapy and radiation therapy.⁹⁹ Therapeutic efficacy of anti-cancerous drug depends on their precise delivery with a minimal side effect to healthy tissue. Here, CNTs precisely single walled carbon nanotubes (SWCNTs) are key

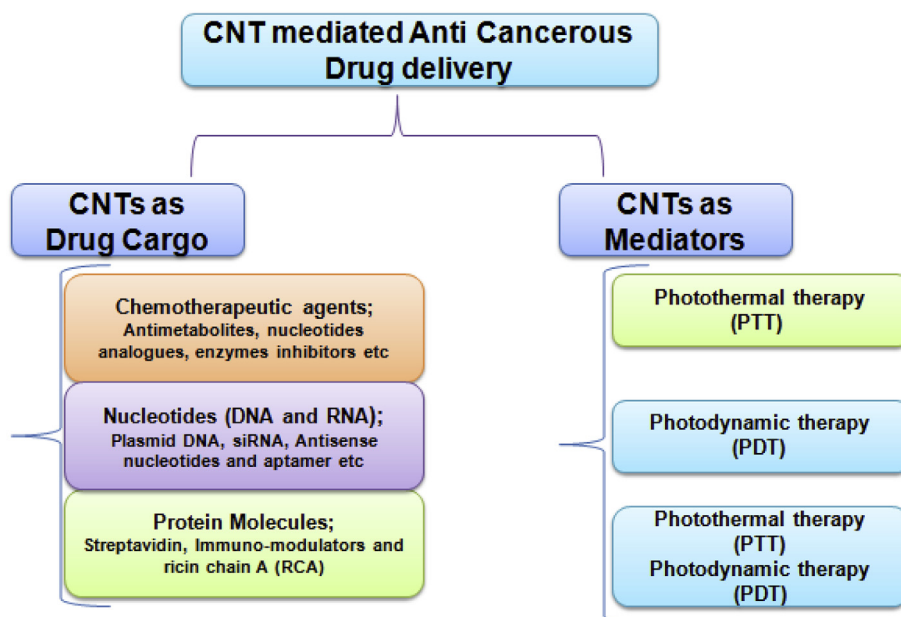


Figure 2 The figure demonstrates CNT mediated anti-cancerous drug delivery. The figure summarizes various approaches for carbon nanotubes (CNT) mediated anti cancer drug delivery. CNT can be use as drug cargo carrying single or multiple anti cancer drug and CNT as a whole conjugated with particular functional groups act as mediator for anticancer therapy.

drug delivery vehicles for anti-cancerous drug delivery.¹⁰⁰ The functionalized SWCNTs are an ideal choice for delivery of chemotherapeutic agents, nucleotides, and protein molecules. Studies have demonstrated that SWCNTs are not only effective in delivery anti-cancerous drugs but also accelerate the killing of cancer cell via their photothermal effects.¹⁰¹ Liu et al, 2008 have demonstrated functionalized SWCNTs are effective in targeting tumor cell and biocompatible with minimal side effects. The photothermal capacity acts as an additive in cancer treatment and SWCNTs carrying photothermal property which can be further optimized by functionalization.¹⁰² There are several studies demonstrated the potential of SWCNTs is not limited to the drug delivery but also reported in real-time imaging. The molecular etiology of cancer including breast cancer is highly complex and involves abnormal functioning of several metabolic regulators (protein and nucleotides) and hence targeting these molecular switches is an effective way for cancer management.¹⁰³

CNTs, including both SWCNTs and multiwalled carbon nanotubes (MWCNTs), have shown promising results in delivering exogenous protein and nucleotides as negative regulators. Zhao et al, 2005 succeeded in synthesizing water-soluble SWCNTs with enormous potential in drug delivery system, including anticancer drug delivery.¹⁰⁴ SWCNTs with significant water solubility provide higher affinity to plasma protein and biocompatibility. Subsequently, Chen et al, 2008 synthesized SWCNTs capable of dispersion in aqueous solution and controlled by light.¹⁰⁵ All these development in SWCNTs had shown tremendous scope in controlled drug delivery and real-time monitoring of the fate of drug delivery. The CNTs, including both SWCNTs and MWCNTs, are an effective carrier for drug delivery to the neuronal tissue across the blood–brain barrier.¹⁰⁶ Additionally, CNTs plays a vital role in the real-time monitoring

of clot lysis and the effectiveness of the implemented drug against thrombotic vascular disorders.¹⁰⁷ The CNTs extensively used in the biomarker development for inflammatory and autoimmune disorders.¹⁰⁸

Applications of MWCNTs

Multiwalled carbon nanotubes (MWCNTs) are well ordered, hollow, carbon graphitic nanomaterials with a range of properties. MWCNTs have shown tremendous scope in biomedical applications and transformed as potential drug delivery vehicles for anti-cancerous drug delivery.¹⁰⁹ Additionally, appropriate functionalization offers additional properties ideal for drug delivery. Filling MWCNTs with an appropriate anticancer drug is another method of delivering anticancer therapy.¹¹⁰ MWCNT possesses 80 nm diameters to accommodate nearly 5 million candidate drug molecules.¹¹¹ During the process of functionalization incorporation of appropriate molecules, a mostly functional group to the CNT surface allows these materials less toxic and more biocompatible.¹¹² In the present scenario, the MWCNTs research is more likely to involve a reduction in toxic properties using functionalization process. This is a desirable feature and needs further research to avoid the problem of toxicity.¹¹³ The amount of nanoparticles entering the body also has a major impact on toxicity. The current research in MWCNT in context with drug delivery largely focuses on the reduction of MWCNTs based toxicity. Fan et al 2006 have developed MWCNTs conjugated with Al_2O_3 composite with diverse biomedical applications including drug delivery vehicles.¹¹⁴ MWCNTs are vital in analyzing inflammatory diseases and their diagnosis. Several biomarkers have been developed using MWCNTs and shown a promising result in animal studies.

Development in SWCNTs for anti-cancerous drug delivery

Over a period, SWCNTs extensively used as a potential carrier for anti-cancerous drug delivery and achieved tremendous success. The animal studies using anti-cancerous drugs conjugated with SWCNTs have shown a promising result. The unique physicochemical properties (optical and magnetic) of SWCNTs offer a wide range of application in cancer therapeutics including drug delivery, accelerated killing of the tumor cells, and early diagnosis.¹¹⁵ The functionalized SWCNTs render side effects and associated complications however, clinical trials studies are underway. Additionally, SWCNTs had shown exponential growth in the development of diagnostics for early detection of various types of tumors. In a recent study, Faraj et al, 2016 investigated potential of SWCNTs for specific targeting and noninvasive imaging of breast cancer.¹¹⁶ Here, in the study, SWCNTs functionalized with Polyethylene glycol and conjugated with CD44 antibodies for real-time detection. Nima et al, 2013 investigated potential of SWCNTs in drug delivery and robust diagnosis of single human breast cancer cell via Raman spectroscopic analysis.¹¹⁷ Mohammadi et al, 2015 synthesized SWCNTs functionalized with aptamer and, piperazine-polyethyleneimine derivative for efficient tagging of siRNA. In the study, using antisense technology mediated by siRNA proliferative capacity of breast cancer cell was inhibited and shown a scope for future medicine.¹¹⁸ The studies also have shown that SWCNTs alone is effective as the anti-cancerous drug and Zhou et al, 2011 demonstrated mitochondria targeting and killing of the cancer cells via photothermal effect.¹¹⁹ Table 1 summarizes potential of SWCNTs as carrier for anti cancer drug for the treatment of various cancers.

Development in MWCNTs for anti-cancerous drug delivery

The physicochemical properties of MWCNTs make them unique and capable of changing the biological or toxicological behavior of living organisms or the environment.¹²⁰ Pantarotto et al succeeded in developing functionalized CNT-DNA complexes and reported high DNA expression compared with naked DNA.¹²¹ The structural insights reported crucial in cellular uptake of CNTs by cells. In a study

intended to deliver CNTs to HeLa Cells, the perpendicular positioning of MWNTs was reported more effective during the internalization process.¹²² It is mainly the mechanism to deliver targeted effects of CNTs like drug release and diagnosis intended to cancerous cells which enable CNTs and drug conjugates during the process of internalization. However, the release of drug from the CNTs—drug conjugate is also important to ensure the efficacy of the drug delivery system.¹²³ Considering physiological events, MWNTs reported with an increased influx of immune cells precisely macrophage into glioma cells increase tumor cytokine level (IL-10). Handei et al. 2009. MWNTs were also effective in delivering proteins molecules in the cancerous tissue.¹²⁴ Weng et al, 2009. Although effective CNT based protein delivery evaluated in vitro and in vivo system, the clinical application of these approaches remains questionable. It is mainly due to the safety issue of CNTs which have not addressed adequately. Additionally, several other parameters such as targeting cancer cells and controllability are required to be addressed to have effective use of MWCNTs in drug delivery.¹²⁵ Over the period, several factors have been optimized such as purification and functionalization of CNTs to enhance safety. Further, the CNTs often used in studying the enzyme—substrate complex formation and their affinity. Hence, the size of synthesized CNTs and drug load in CNTs drug complex must be uniform.¹²⁶ It was also reported that the delivery of enzyme and protein in conjugation with CNTs enhances the effectiveness of therapeutic biomolecules. Table 2 summarizes potential of MWCNTs as carrier for anti cancer drug for the treatment of various cancers.

Scope and prospects of CNTs

The CNTs due to their unique structure, physiochemical, optical and magnetic properties gained attention in several fields including environment, agriculture, electronics, and biomedical as well. Both SWCNTs and MWCNTs have their advantages and disadvantage extensively used in biomedical applications.¹²⁷ There is a great demand for the use of these tiny structures in the diagnosis and drug delivery vehicles. Additionally, the physiochemical, optical and magnetic properties were studied for their therapeutic potential in several diseases models.¹²⁸ In future CNTs will be an ideal choice for drug targeting and delivery against

Table 1 Potential of SWCNTs as drug cargo for anti-cancerous drug delivery.

CNTs	Cancer Type	Drug	Highlights
SWCNTs	Leukemia, breast and ovarian cancer	Doxorubicin	The SWCNTs conjugated with Doxorubicin has shown reduced drug dependent toxicity in animal studies
SWCNTs	Prostate cancer, and acute nonlymphocytic leukemia	Mitoxantrone	The Mitoxantrone conjugated with SWCNTs has shown reduced drug dependent toxicity in animal studies
SWCNTs	Breast Cancer	Paclitaxel	The drug Paclitaxel tagged with SWCNTs reported a higher $t_{1/2}$ with the slower renal and hepatic clearance
SWCNTs	Ovarian cancer, cervical cancer, and breast cancer	Cisplatin	A reduced drug dependent toxicity was reported in animal studies if Cisplatin was conjugated with SWCNTs
SWCNTs	Ovarian cancer, lung cancer, and neuroblastoma	Carboplatin	SWCNTs conjugated Carboplatin showed a significant reduction in tumor cell proliferation

Table 2 Potential of MWCNTs as drug cargo for anti-cancerous drug delivery.

CNTs	Cancer Type	Drug	Highlights
MWCNTs	Breast cancer	Doxorubicin	Doxorubicin and Pluronic-MWCNT used in an equal volume of doxorubicin hydrochloride with increasing MWCNT aqueous dispersion concentrations CNT suspension in carboplatin solution (10 mg/mL).
MWCNTs	Bladder Cancer	Carboplatin	Amine-MWCNTs generated through 1,3-dipolar cycloaddition reaction of azomethine ylides.
MWCNTs	Breast cancer	Methotrexate	
MWCNTs	Lymphoma	Doxorubicin	supramolecular π - π stacking to load a cancer chemotherapy agent doxorubicin onto branched polyethylene glycol functionalized CNTs for in vivo drug delivery applications
MWCNTs	Human gastric carcinoma	HCPT	HCPT is linked the to MWCNTs using diaminotriethylene glycol (hydrophilic spacer) biocleavable ester linkage
MWCNTs	Leukemia	Amphotericin B	Carboxylated CNTs were, treated with $[\text{NH}_2(\text{CH}_2)_2\text{NH}_2]$, forming amine groups on the CNT surface.
MWCNTs	Breast Cancer	Paclitaxel	Paclitaxel conjugated to branched PEG chains on SWCNTs via a cleavable ester bond to obtain a water-soluble SWCNT-paclitaxel

various diseases including cancer. However, there will be more emphasis on cancer drug delivery and involvement of CNTs as a potential drug carrier.¹²⁹ Due to their size (nanoscale), intracellular trafficking and movement across the subcellular compartment result in CNTs excellent carrier not only for chemotherapeutic agents but also biomolecules including protein and nucleotides.¹³⁰ The CNTs are in clinical application for precise diagnosis of disease and real-time monitoring of drug delivery.

Functionalized CNTs both SWCNTs and MWCTNs with predefined physiochemical, optical and magnetic properties will offer a more effective diagnosis. The use of CNTs will be more centric to tissue engineering and the development of the artificial organ in the future. The unique atomic arrangement in CNTs provides an ideal scaffold for cellular growth into tissue and organ.¹³¹ Despite the vast application of CNTs, several drawbacks and limitation are needing immediate attention to overcome for their robust uses. The side effect and toxicity of CNTs depends on several factors including the method of synthesis, purification, and presence of the functional group.¹³² There is an extensive research work underway to surmount these limitations of CNTs and hope in future CNTs will be playing a vital role in biomedical engineering. However, modulating CNTs physiochemical properties and translate them into drug cargoes remain a major challenge. Over time, nanotechnology and incorporated changes into CNTs time associated with the serious tissue and cellular toxicity.

Conclusion

Cancer remains a leading cause of mortality and physical deformity worldwide. The rise and prevalence of breast cancer posed a new challenge to therapeutics. Lack of precise therapeutics and drug delivery systems lead to cancer incurable. There is a close association between CD44 including isoforms and cancer including the extent of metastasis. A preponderance of research data demonstrated that CD44 overexpression and transition among its various isoforms are closely associated with various forms of cancer. Additionally, several therapeutic have been developed targeting CD44 along with its isoforms variants

using monoclonal antibodies, mimetic peptides, or more recently, microRNA. For breast cancer, targeting folate receptor and hyaluronic acid showed increasing potential for future cancer therapeutics. Now, using the potential of CNTs, these therapeutics gained tremendous success in drug delivery diagnosis development and anti-cancerous therapeutics. However, these nanodesigns are also associated with several limitations mainly toxicity. The CNT toxicity is function of nature, size, chemical synthesis, purification, and functionalization as well. The toxicity can be reduced via surface functionalization and purification approaches. The appropriate surface functionalization not only reduces toxicity but also enhance specificity towards targeted receptor. CNTs are ideal choice for modern cutting edge imaging techniques for cancer management. Functionalized CNTs such as folic acid conjugated CNTs shows effective imaging in magnetic resonance imaging for breast and other tumors. The CNTs are key biomaterial extensively use in tissue engineering and organ growth for clinical transplantation. The surface functionalization also enhances the biocompatibility of CNT to the selective cells and tissue. To enhance biocompatibility and toxicity of CNTs one major surface functionalization approach is creating hydrophilicity of formulation. The HBL system becomes important here to ensure biocompatibility and toxicity of CNTs. The surface functionalization of CNTs outlooks mainly the higher water-soluble property of CNTs which lowers the toxicity and higher biocompatibility.

Conflict of interest

The author declares no conflict of interest.

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