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REVIEW / DERLEME

Cd44 Targeted Plga Nano-Medicine For Cancer Chemotherapy- A Comprehensive Review

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Abstract

In order to deliver therapeutic agents to tumour tissues more specifically, the scientific community has focused a lot of attention recently on unravelling the mystery of cluster of differentiation-44 (CD44). Additionally, drug delivery researchers are interested in using nanomedicines to target this receptor because of its over-expression in a variety of solid tumors. Conventional nanomedicines based on biodegradable polymers such as poly (lactide-co-glycolide) (PLGA) are often associated with insufficient cellular uptake by cancer cells, due to lack of active targeting moiety on their surface. Therefore, to address this limitation, CD44 targeted PLGA nanomedicines has gained considerable interest for enhancing the efficacy of chemotherapeutic agents.

We have thoroughly covered the most recent developments in the design and synthesis of CD44-targeted PLGA nanomedicines in this review, which are being used to enhance tumor-targeted drug delivery. Additionally, we have talked about employing PLGA-based nanomedicines to co-target CD44 with additional targeting molecules such folic acid, human epidermal growth factor 2 (HER2), and monoclonal antibodies. Recent research on poly (lactic-co-glycolic acid) encapsulated platinum nanoparticles for the treatment of cancer was also covered in this review. We talk about the role that newly created nanomedicines can play in enhancing the efficacy and PK of existing therapy regimens. We offer insight into the development of more potent therapeutic regimens to enhance the clinical outcomes of cancer treatments by explaining the state-of-the-art of nanomedicine and analyzing their clinical benefits and problems.

Keywords: Nanomedicine, CD44, PLGA, Anticancer, Nanoparticle, Hyaluronidase

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INTRODUCTION

Chemotherapy is the cornerstone of cancer treatment, which also includes a variety of surgical techniques, radiotherapy, antibody therapy, hormone therapy, and chemotherapy. Cancer is one of the deadliest diseases (1,2). However, the poor aqueous solubility of chemotherapy drugs (which makes them difficult to administer), non-specific distribution, a limited therapeutic index, and efflux transporter specificity (which confers drug resistance in cancer cells and reduces drug bioavailability) are the main drawbacks of cancer chemotherapy (3,4).

Because of their high surface-to-volume ratios, nanoscale sizes, and desirable physicochemical properties, nanocarrier-based delivery systems have attracted a lot of research attention from scientists looking for ways to overcome these obstacles. These systems have the potential to enhance the therapeutic effects of chemotherapeutic agents (5,7,8,11). Chemotherapeutic agents can be delivered to a targeted spot using nanocarrier drug delivery systems, which can also change the pharmacokinetics and tissue bio distribution profile of the drugs. Additionally, these nanocarriers can successfully penetrate into cancer cells by "passive targeting" due to the leaky vasculature of malignant endothelium cells; this phenomenon is known as the "Enhanced Permeation and Retention (EPR)" effect (12).

To increase the bioavailability and effectiveness of chemotherapeutic drugs, numerous types of nanocarriers, including inorganic nanoparticles (NPs), viral NPs, lipid-based NPs, and polymer-based NPs, have been thoroughly investigated. Due of their biodegradable and biocompatible characteristics, polymer-based NPs, particularly those based on PLGA, have received the most attention among these nanocarriers (5,7,8). Although polymeric nanocarriers showed passive targeting to tumours, they have a number of drawbacks, such as the fact that not all cancers can be targeted with the EPR effect since the permeability of the blood arteries varies depending on the kind of tumour (13,14,15).

Due to the expression and presence of cell surface receptors or epitopes, active targeting based approach has been used to overcome these limitations. Nanocarriers can be attached with affinity ligands such as antibodies, peptides, aptamers, or small moieties that bind to only tumour cells through ligand-receptor interactions. In this context, the stem-like cell receptor CD44, also known as H-CAM, Hermes Ag, and human phagocytic glycoprotein-1, has attracted a great deal of research attention as a possible target against a variety of malignancies, including pancreatic, breast, ovarian, prostate, colorectal, and neuroblastoma (16,17). It has been discovered that the CD44 receptor activates a number of cancer signalling pathways, including the Pi3K/ Akt, Ras MAPK, and Rho GTPase pathways, which promotes cancer cell growth, invasion, and angiogenesis. Drug delivery experts have therefore concentrated their considerable emphasis on designing CD44 targeted PLGA formulations to transport the chemotherapeutic medications specifically towards tumours, taking into account the benefits of CD44 receptors as well as PLGA based formulations (18,19,29). In order to create CD44-targeted PLGA nanomedicines, drug delivery researchers have developed a variety of techniques. However, special attention has also been paid to the main effects of these nanomedicines utilised to boost the effectiveness of chemotherapeutic drugs. It is anticipated that this review article will be helpful in directing future research efforts and motivating a large number of researchers to take up difficulties in this new area of study.

2. PLGA Based Nano Carriers For Drug Delivery

Because of its biodegradability (degrades in vivo into non-toxic lactic acid and glycolic acid), biocompatibility, and Food and Drug Administration (FDA) approval for clinical usage, PLGA has been the material of choice for drug administration and as scaffolds in tissue engineering. The PLGA-based nanocarriers can deliver chemotherapeutic drugs with prolonged drug release, and they are easily adaptable to offer stealth and efficient biological interactions (36,38,42).

Table: 1, PLGA based formulations by the FDA:

S.no	Formulations
1.	Arestin®
2.	Trelstar®
3.	Profact®
4.	Decapeptyl®

The significance of PLGA based nanocarriers in biomedical sciences has been critically reviewed in several reviews (42) and therefore detailed discussion in this area is beyond the scope of this manuscript.

3. CD44 Gene And Protein Structure

The term "CD44" refers to a group of single-chain, single-pass, and trans-membrane glycoproteins that are all encoded by a single, roughly 50 kb-long gene on human chromosome 11. The CD44 gene has 20 exons, the first five (1-5) and last five (16-20) of which are constant and expressed in all isoforms of the protein. Between these regions, the ten exons (6–15) are subject to alternative splicing, which produces a variety of CD44 variants (CD44v), some of which can be further changed by N- and O-linked glycosylations (37,54).

The membrane-proximal stem region is where the 10 core exons, often referred to as "variant" exons (v1-v10), are excised or spliced to create different combinations. Three functional domains make up the proteins produced by the CD44 gene: an extracellular N-terminal domain, a trans membrane region, and a carboxyl-terminal cytoplasmic tail (49). (Figure 1) (76).

The CD44 isoform without variant exons, the standard CD44 (CD44s), is highly expressed in both normal and tumor cells, whereas the variant CD44 (CD44v) isoforms are expressed in a variety of different cancers, particularly in advanced stages. Among CD44v isoforms, CD44v6 have shown the crucial role in cancer progression because of its ability to bind hepatocyte growth factor (HGF), osteopontin (OPN), and other key cytokines which are produced by the tumor microenvironment. In addition to this, CD44v2, CD44v3, CD44v5, CD44v9, CD44v10, and CD44v8–10 have also been demonstrated to have the important role in different cancers (28). For example, CD44v9 expression in primary early gastric cancer is a prognostic marker for recurrence and the existence of CD44v9-positive circulating cancer cells is often related with refractory disease, recurrence, and reduced survival rates in colorectal cancer (34). The detailed discussion of CD44 structure has been described in several reviews (43,76) and a detailed discussion in this area is beyond the scope of this manuscript.

The CD44 is the major receptor for hyaluronan on the cell surface, and the binding of hyaluronan to CD44 induces signaling (such as ErbB2, EGFR, PDGFRβ, and TGF-beta) and leads to tumor progression (29,43,45). The binding of CD44 to HA is located on the constant N-terminal part of CD44 and therefore present on all CD44 isoforms (Cortes-Dericks and Schmid, 2017), However, HA-binding capacity is not a constant feature of CD44, but it is subject to complex regulation involving several mechanisms such as alternative splicing, modulation of cytoskeletal interaction, and posttranslational modification of CD44 (43).

Recently, Vuorio et al. demonstrated three different types of binding for the CD44-HA interaction using atomistic MD simulation data (69). The three binding modes were named `crystallographic', `parallel', and `upright' modes. The relative binding affinity studies demonstrated crystallographic mode is the strongest among three modes.

4. Therapeutic Strategies For Targeting Cd44 In Cancer Chemotherapy

The over-expression of CD44 in several cancers, such as pancreatic, lung, ovarian, breast, leukemia, etc., has made it a promising target for cancer drug delivery (35,). Further, there is evidence that there is gain in CD44 expression in the breast cancer cells when they acquired resistance to tamoxifen (27). Hence, there is need of novel therapeutic strategies that could selectively target such cancerous cells which over express CD44 in order to improve the efficacy of chemotherapeutic agents.

The strategies which have been commonly used to target CD44 are the use of (a) HA oligomers which inhibit the binding of high molecular weight HA to CD44 (57,62) (b) anti-CD44 monoclonal antibody and antibodies against CD44v6 (26); (c) the use of CD44v6 peptides in order to block CD44v6-c-Met interaction (41); (d) the use of CD44v10 aptamers in order to prevent complex formation with the surface protein EphA2 (accounts for migratory ability of cells) (25); (e) enzymatic hydrolysis of HA by hyaluronidase cleaves high molecular weight HA into smaller fragments and thus prevents HA-CD44 interaction (37) and (f) short hair pin RNA to down regulate the expression of CD44 (43). These strategies have been extensively reviewed earlier and therefore, a comprehensive discussion in this field is beyond the scope of this manuscript (43,37). The interaction of CD44 with HA, monoclonal antibodies, aptamers etc. has been exploited by drug delivery researchers in order to deliver the chemotherapeutic drugs selectively towards tumor tissues. The CD44 targeting ligands which have been mostly been used by drug delivery researchers are described in following sections.

4.1 Hyaluronic Acid

Hyaluronic acid (HA) is a non-toxic, non-immunogenic, water-soluble and linear anionic polysaccharide. It is negatively charged at physiological pH due to the dissociation of the carboxyl group of d-glucuronic acid (24,72). At the molecular level, HA interacts with cell surface receptors mainly with CD44 and RHAMM (receptor for hyaluronan mediated motility) and also with other receptors such as ICAM-1 (Intercellular Adhesion Molecule 1), LYVE-1 (lymphatic vessel endothelium receptor-1). Due to the capability of HA towards binding to CD44 receptors, HA-based nanocarriers have been explored to target CD44-overexpressing malignant cancer cells for delivery of chemotherapeutic agents (50,10). In addition to this, the presence of multiple reactive functional groups (hydroxyl and carboxylic acid), HA can be conjugated with hydrophobic as well as hydrophilic anticancer agents (10). It has also been demonstrated that high molecular weight HA (>200 kDa) is often associated with a shorter half-life (t1/2) in vivo and clears from systemic circulation due to HA receptor for endocytosis present in lung and liver (32).

4.2 Chondroitin Sulfate

Chondroitin sulfate (Chs), a naturally present anionic glycosaminoglycan, consists of repeating unit of a disaccharide having β-1,4-linked d-glucuronic acid (GlcA) and β-1,3-linked Nacetyl galactosamine (GalNAc) sulfated at either 4 or 6 positions. It has been reported to specifically bind to CD44 receptors and thus facilitates the higher uptake of nanoparticles inside the cells (29,77).

4.3 Anti-CD44 Antibodies

The use of anti-CD44 antibodies to target and block the CD44 receptor on cancer cells is an excellent approach for targeted delivery. It has been demonstrated that anti-CD44 antibodies can inhibit cancer progression and ability to induce cell differentiation or apoptosis in leukemic cells (22,39). These antibodies can be attached to radioisotopes, chemotherapeutic drugs, toxins and on to the surface of the nanoformulations. Based on this concept, Bivatuzumab (BIWA-4, mAb against CD44v6) along with microtubule inhibitor mertansine was developed. However, its phase I trial was disappointing due to severe skin toxicities were observed in mertansine conjugates (56).

4.4 Aptamers

Aptamers are small synthetic DNA, RNA or peptides molecules that bind to specific protein targets (similar to mAbs), can easily be attached and decorated on to the surface of nanocarriers for active targeting against CD44 (6,46). These aptamers have advantages over antibodies due to their easy tagging procedure with various reporters for molecular recognition, the ability of directional PCR amplification and low cost of production (46). In a recent study by Alshaer et al. conjugated anti-CD44 targeting aptamer (Apt1) with PEGylated liposomes using the thiol–maleimide click reaction (6). Their study demonstrated the conjugation of anti-CD44 aptamer to the liposome surface enhanced the selectivity of liposomes in CD44 over-expressing human lung cancer cells (A549) and human breast cancer cells (MDA-MB-231) as compared to non-targeted liposomes.

Table: 2Cancer nanomedicines undergoing Phase II/III clinical trials-er nano

Product	Active components	NP size	Trial phase
Genexol-PM1	PTX	23.9 nm	II/III
NK-1051	PTX	85 nm	III
NC-6004	Cisplatin	30 nm	II/III
LipoplatinTM	Cisplatin	110 nm	II/III
CriPec1	Docetaxel	65 nm	I/II
CRLX101	Camptothecin	30 nm	II
NanoTherm	Aminosilane- coated SPIONs	15 nm	I/II; marketed in EU
ThermoDox	DOX	-	II/III
NBTXR3	Coated hafnium oxide NPs	50 nm	II/III
SGT-53	Human wild-type p53 DNA	-	II
GEN-1	IL-12 plasmid	-	I/II

5. CD44 targeted poly (lactide-co-glycolide) nanomedicines

Among various ligands used for CD44 targeting, hyaluronic acid has been the most commonly exploited to design these nanomedicines. In this review, we have categorized the strategies used for designing CD44 targeted PLGA nanomedicines into two parts, i.e. (a) hyaluronic acid decorated PLGA nanocarriers and (ii) miscellaneous nanocarriers. These strategies are described in the following sections.

5.1 Hyaluronic Acid Decorated Plga Nanocarriers

Hyaluronic acid targeted PLGA nanomedicines encapsulating a number

of chemotherapeutic drugs have been developed in order to achieve targeted delivery towards CD44 over-expressing cancer cells (Table 1). The hyaluronic acid can be anchored on to the surface of PLGA nanocarriers either through conjugation or electrostatic interactions (Figure 2b). In electrostatic interactions, initially, PLGA nanoparticles are prepared using positive charge surfactants such as didodecyldimethylammonium bromide (DMAB), cetyl trimethylammonium bromide (CTAB) and then functionalized with HA using its anionic nature (Figure 2c). The conjugation of CD44 HA with PLGA has been designed using several linkers such as poly (ethylene) diamine, 1,4- diaminobutane, cystamine, adipic dihydrazide etc. (Figure 2d). Some of the linkers used for conjugation are redox sensitive (e.g., cystamine) and therefore degraded under a specific environment and thus provides site-specific delivery.

5.1.1 Conjugation Based Strategies

The conjugation based approaches have been widely exploited for CD44 targeted PLGA nanoparticles. Various HA grafted PLGA co-block polymers have been synthesized using different linkers and formed into nanoparticles using dialysis, nanoprecipitation or solvent evaporation methods. For example, Yadav et al. synthesized hyaluronic acid-poly(ethylene glycol)- poly(lactide-co-glycolide) (HA-PEG-PLGA) tri-block copolymer and prepared doxorubicin (DOX) nanoparticles using nano precipitation method (74). The poly (ethylene glycol) di amine was used as a linker for conjugation of carboxylic group of HA and PLGA carboxylic group. In vivo studies in Ehrlich ascites tumor (EAT) tumor model demonstrated higher accumulation of DOX in the tumor tissues in case of HA-PEG-PLGA-DOX nanoparticles as compared with untargeted nanoparticles and native DOX. In another study, 5-Fluorouracil (5-Fu) nanoparticles were developed using the similar method (74). In vitro cytotoxicity studies in EAT cell lines demonstrated enhanced cytotoxicity of HA-PEG-PLGA-FU nanoparticles as compared to native 5-FU. The superior in vitro antitumor efficacy was also supported by in vivo studies in EAT tumor model. The HA-PEG-PLGA-FU nanoparticles suppressed the tumor growth more significantly (4.6-times) than the animals treated with native 5- FU in solution. The enhancement in therapeutic efficacy was attributed due to target specific delivery due to HA and enhanced permeation and retention effect.

Lee et al. synthesized PLGA-grafted HA copolymers by using the PEG-assisted solubilization method in anhydrous dimethyl sulfoxide (DMSO) (29). The developed co-polymer demonstrated self-assembly in aqueous solution to form core/shell type micellar aggregates, and DOX was entrapped during the self-assembly. The study demonstrated DOX loaded HA-g-PLGA nanoparticles enhanced the cellular uptake and cytotoxicity against HCT-116 cells as compared to native DOX due to over-expression of the CD44 receptor. Huang et. al. synthesized a series of PLGA/HA block copolymers of different molecular weight using an end to end coupling strategy and prepared docetaxel (DTX)-loaded self-assembled NPs (SANPs) using these block co-polymers (Figure 3) (20). The cellular uptake studies demonstrated SANPs uptake in MDA-MB-231 cells was mainly through CD44-mediated endocytosis via an energy-dependent endocytic pathway. The in vitro cytotoxicity studies demonstrated enhanced cytotoxicity of DTX/SANPs as compare to native DTX in MDA-MB-231 cells due to over-expression of CD44 receptors. However, this difference was insignificant in case of MCF-7 cell lines due to low expression of CD44. The in vivo pharmacokinetic studies after

intravenous injection demonstrated the longer circulatory behavior of DTX/SANPs as compare to native DTX. The long circulatory behavior of the NPs also resulted in superior in vivo efficacy of DTX/SANPs as compare to native DTX in MDA-MB-231-bearing female nude mice.

The PLGA and HA has also been covalently conjugated using stimuli responsive linkers. For example, in an investigation hyaluronic acid-cystamine-polylactic-co-glycolic acid (HA-SSPLGA) was synthesized by grafting PLGA onto HA using the linker molecule cystamine, a disulfide-based bifunctional primary amine (19). The disulfide linker acts as a bio reducible linker and can be broken by glutathione, which is more concentrated within the intracellular spaces in cancer cells. The synthesized co-polymer was used to co-deliver DOX and cyclopamine (CYC, a primary inhibitor of the hedgehog signalling pathway of CSCs) to both CD44-overexpressing breast CSC subpopulation and bulk breast cancer cells and permit an on-demand release. The study demonstrated the dual drugs-loaded HA-SSPLGA NPs demonstrated enhanced inhibition to the tumor sphere formation as compared with untargeted PLGA particles. In another investigation, HA and PLGA co-polymer has also been synthesized using hexa methylene diamine (HMDA) linker to deliver DOX via formation into self-assembled micelles (79). The intracellular uptake studies demonstrated enhanced cellular uptake in CD44 positive HepG2 cells due to receptor mediated endocytosis.

Recently, HA-grafted-PLGA copolymer were synthesized which has the ability to self assemble in aqueous solution because of amphiphilic nature and thus resulting in formation of nanoparticles (21). The dietary molecule, bromelain was loaded into the nanoparticles to improve its anti-tumor efficacy and selective delivery towards CD44 over expressing cancer cells. The cellular uptake studies demonstrated higher uptake of developed NPs towards CD44 over-expressing MCF-7 cells as compared to A549 cells. Further, the in vivo efficacy studies in tumor-bearing mice demonstrated that HA targeted NPs exhibit superior efficacy and were efficient in suppressing the tumor growth as compared to native bromelain.

One of the significant limitations faced in preparation of nanoparticles using his co-block polymers is the poor solubility of the synthesized co-polymers in organic solvents. Therefore, to address this problem, some researchers, have conjugated the targeting ligands after preparation of the nanoparticles. For example, in an investigation, SN-38-loaded hyaluronic acid (HA)-decorated poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) nanoparticles (NPs) for targeted delivery for ovarian cancer. In this study, initially, PLGA-PEG co-polymers were developed and SN-38 loaded nanoparticles were developed using emulsion-solvent evaporation method. After preparation of nanoparticles, HA was conjugated to the surface of the NPs via EDC/NHS chemistry. In vitro cellular uptake studies demonstrated 8 and 16 fold higher uptake of HA functionalized NPs in CD44-positive cell lines, SKOV-3 and OVCAR-8, compared to CD44-negative cells (CHO). The in vitro cytotoxicity was in also the correlation with the cellular uptake and for CD-44 over expressing cancer cell lines, targeted NPs cytotoxicity was significantly higher as compared to non-targeted NPs. Recently, we have developed HA-conjugated PLGA-PEG NPs for delivery of a novel thiotetrazole analog of IC87114 (PI3Kδ inhibitor) for its delivery towards CD44 over-expressing cancer cells (2). The in vitro cytotoxicity and intracellular uptake studies demonstrated enhanced cytotoxicity and accumulation of HA conjugated PLGA-PEG NPs as compared to PLGA-PEG NPs in high CD44 expressing MiaPaca-2 cells as compared to MDAMB-231 and MCF7 cells. The mechanistic studies

revealed HA conjugated PLGA-PEG NPs were able to induce premature senescence with increase in senescence-associated β-galactosidase activity and senescence specific marker p21 expression through modulation of Pi3K/Akt/NF-kB signaling pathway in MiaPaca-2 cells.

The CD44 targeted PLGA NPs have also been developed for triple negative breast cancer (TNBC) therapy. TNBC refers to any breast cancer that does not express the molecular markers for progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2). For example, in a recent study, hyaluronic acid (HA)-decorated polyethylenimine-poly(D,L-lactide-co-glycolide) (PEI-PLGA) nanoparticles were investigated for co-delivery of DOX and miR-542-3p for TNBC therapy (63). MiR-542-3p serves as a novel regulator of the p53 tumor suppressor molecule and downregulate the expression of the anti-apoptotic protein surviving (47,63). The intracellular uptake studies demonstrated increased the intracellular fluorescence of both DOX and the FAM labelled miR-542-3p in MDA-MB-231 cells as compared to MCF-7 cells because of 20.5-fold overexpression of CD44 in MDA-MB-231 as compared to MCF-7 cells. The enhancement in cellular uptake also resulted in higher cytotoxicity and apoptosis of HA functionalized nanoparticles as compare to un-modified nanoparticles. In a recent study, HA decorated PLGA NPs of paclitaxel (PTX) by using a novel biocompatible surfactant vitamin E-oligo(methyl diglycol L-glutamate) (VEOEG) (65). The terminal amino groups of VEOEG surfactants allowed the conjugation of carboxylic group of HA onto the surface of the developed NPs. The in vitro cytotoxicity studies in MCF-7 cells demonstrated PTX- HA-PLGA NPs have two-fold lower IC50 than taxol due to CD44 receptor-mediated internalization of NPs. In vivo pharmacokinetic studies after intravenous administration demonstrated the long circulatory behavior of PTX in the developed NPs with having half-life of 3.14 h as compared to native taxol (0.32 h). The in vivo bio distribution of Cy5- labeled HA-PLGA NPs in human MCF-7 tumor-bearing nude mice confirmed that tumor Cy5 fluorescence was stronger as compared to all the healthy organs after 48 h intravenous post injection. These results were also in correlation with in vivo studies in which PTX loaded HA PLGA NPs demonstrated superior efficacy as compared to taxol. In another study by the similar group, DTX loaded HA-PLGA NPs were developed to target orthotropic human lung cancer (65). The results obtained in the study demonstrated enhanced cytotoxicity of developed NPs in CD44+ A549 cells and a prolonged elimination half-life of 4.13 h in nude mice. Further, in vivo efficacy studies also demonstrated superior efficacy of DTX-loaded HA-PLGA NPs in orthotropic human A549-Luc lung tumor-bearing nude mice. The DTX loaded HA-PLGA NPs were also prepared using vitamin E-SS-oligo(methyl diglycol L-glutamate) (VE-SS-OEG) which acts as reductively cleavable surfactant (RCS) (Figure 4) (65). The in vitro and in vivo studies demonstrated DTX-loaded redox-sensitive HA-PLGA NPs possess superior stability, potent antitumor activity, long circulation time and enhanced inhibition of orthotropic human A549-Luc lung tumor in nude mice as compared to native DTX.

5.1.2 Electrostatic Interaction Strategies

The use of electrostatic interaction strategies for designing CD44 targeted PLGA nano-medicines have been described in several reports. In a recent study, paclitaxel (PTX) loaded HA decorated PLGA NPs were developed in order to actively target the drug to a triple negative breast cancer cells (18). The positively charged PLGA NPs were developed using oil-in-water emulsion method and incorporating cetrimide (positive charge) in the aqueous phase. After removal

of the organic phase (dichloromethane), HA modified NPs were developed by surface charge based interaction with negatively-charged HA. The in vitro cytotoxicity studies demonstrated enhanced cytotoxicity of PTX loaded HA decorated PLGA NPs as compared to nontargeted NPs in MDA-MB-231 cells due to receptor mediated internalization of nanoparticles.

In another investigation, HA coated PLGA nanoparticles for delivery of artersunate (ART) for effective delivery towards CD44 over-expressing cancer cells (61). The positively charged PLGA NPs were developed using di dodecyl dimethyl ammonium bromide (DDAB) as cationic surfactant and thereafter coating it with negatively-charged HA-based on surface charge interaction. The study demonstrated ART loaded HA coated PLGA NPs leads to significant reduction in cell viability as well as significant induction of apoptosis in CD44 over expressing cancer cells as compared to ART loaded PLGA NPs. HA decorated PLGA NPs have also been explored for the co-delivery of therapeutic agents. For example, in an investigation DOX and CPT were co-delivered to inhibit the activity of topoisomerases II and I, respectively, thereby fighting against the cancer stem-like cells (CSC) drug resistance (77). The HA was used as a stabilizer instead of poly (vinyl alcohol) (PVA) in order to achieve targeting effect towards CD44 over-expressing cancer cells. The study demonstrated HA coated PLGA NPs have higher efficacy against both the prostasphere (prostate cancer cell spheroids enriched with CSCs) and nanosphere (breast cancer cell spheroids enriched with CSCs) cells enriched with CSCs in vitro and CSCs in human breast tumor in vivo. This superior efficacy was achieved due to the synergistic anticancer activities of DOX and CPT as well as targeting ability of HA. In another investigation, HA functionalized PLGA NPs were investigated for the co delivery of docetaxel (DTX) and tanespimycin (17-AAG) using hexadecyl trimethyl ammonium bromide (CTAB) as a cationic surfactant (52). The in vitro cytotoxicity studies demonstrated that the dual delivery of DTX and 17-AAG in HA decorated PLGA NPs exhibited synergistic effect in CD44 and RHAMM (CD168) over-expressing MDA-MB-231 and SCC-7 cells. Further, the HA decorated PLGA NPs demonstrated superior in vivo efficacy than either DTX- or 17-AAG-loaded HA-PLGA NPs in SCC-7 tumor xenograft mouse model.

Recently, HA decorated PLGA NPs for the co-delivery of salinomycin (SLM) and paclitaxel (PTX) were developed using di dodecyl trimethyl ammonium bromide (DMAB) as a cationic surfactant (48). The in vitro cytotoxicity studies in MCF-7 cells demonstrated that combination of HA coated SLM NPs and PTX NPs were more potent than their native solutions. Further, the pharmacokinetic parameters demonstrated improved bioavailability of both the drugs from NPs and confirming their longer circulation periods. Xiao et al. developed HA-decorated PLGA NPs for co-delivery of camptothecin (CPT) and curcumin (CUR) for colon cancer-targeting capability (68). The HA was coated onto the surface of the NPs by surface decoration of chitosan (cationic polymer) and thereafter coating the NPs with negatively charged HA (Figure 5). The study demonstrated HA-CPT/CUR PLGA NPs had excellent colon cancer cell-targeting ability and exhibited synergistic effects against Colon-26 cells.

5.2 Miscellaneous Nanocarriers

As described earlier, use of anti-CD44 antibodies to target and block the CD44 receptor on cancer cells has been an excellent approach for targeted delivery. Considering this fact, CD44 monoclonal antibody decorated PLGA nanoparticles were investigated for cisplatin delivery. The NPs were developed encapsulating

cisplatin in maleimide-polyethylene glycol-Poly(d,l-lactic-coglycolide) nanoparticles and thereafter conjugating the surface of NPs with the CD44 monoclonal antibody (19). The in vitro cytotoxicity studies in CP70 and SKOV-3 cells demonstrated better anti-proliferative ability of cis-encapsulating CD44-PEG-PLGA NPs as compared to free form of cisplatin and PLGA NPs without CD44 conjugation. In recent years, gene therapy has gained considerable attention in cancer chemotherapy due to its ability to silence the expressions of functionally relevant tumor genes (51).

There are several genes which are expressed abnormally in cancer, and co-targeting two or more relevant tumor-associated genes may be an effective strategy than targeting the single gene alone. Considering these facts, Zou et al. prepared PLGA NPs for delivery of short hairpin RNA (shRNA) against focal adhesion kinase (FAK) and CD44. FAK (a 125-kDa non-receptor protein tyrosine kinase), is an important mediator of growth-factor signaling, proliferation, migration, invasion and prominent role in angiogenesis (55). The study demonstrated that knockdown of both FAK and CD44 resulted in tumors with inhibited angiogenesis, reduced proliferation and increased apoptosis against ovarian cancer as compared with knockdown of either gene individually.

5.3 Co-targeting of CD44 Based PLGA Nanomedicines

The co-targeting of CD44 with other targeting ligand has emerged as potential strategy to increase differentiation between cancer and normal cells and may be exploited to deliver the chemotherapeutic agents more selectively to cancer cells (29,53,60). In a recent study, Qiao et al. dual targeted PLGA-PEG nanoparticles by conjugating hyaluronic acid (HA) and grafting the doublecortin-like kinase 1 (DCLK1) monoclonal antibody on to their surface (71). The developed NPs have ability to specifically target CD44 receptors and the DCLK1 surface marker, which has ability to distinguish between cancer stem cells (CSCSs) and normal stem cells (NSCs). The in vivo studies in nude mice bearing 4T1 tumors increase in amount of red fluorescence after 12 h at the tumor site in rhodamine loaded DCLK1–HA–PEG–PLGA NPs as compared to untargeted PEG-PLGA NPs after intravenous injection. In another study, Yang et al. developed dual-targeting hybrid nanoparticle (NP) system to deliver SN38 agent specifically to human solid gastric cancer (GC) which have high expression of both human epidermal growth factor receptor 2 (HER2) and cluster determinant 44 (CD44). The hybrid PLGA NPs were prepared by conjugating the anti-Her2/neu AHNP peptide (FCDGFYACYKDV) and n-hexadecylamine (HDA) to the carboxyl groups of hyaluronic acid (HA) (Figure 6). The in vitro cytotoxicity studies revealed that the dual-targeting hybrid NPs could down-regulate the expression of both CD44 and HER2, and thereby hamper the relative signaling cascades and ultimately result in better inhibition of HGC27 cells (gastric cancer cells) growth and invasive activity. Further, in vivo studies in HGC27 tumor xenografted in nude mice demonstrated superior efficacy of the targeted NPs as compared to un-targeted NPs and irinotecan (CPT-11).

Table: 3 Clinically approved cancer nano-medicines y approved cancer nano-medicines

Product	Drug	Carrier components	NP Size	Manufacturer	Approved (year)	Ref.
Doxil1	DOX	Liposome: HSPC, cholesterol, mPEG-DSPE (3:1:1 weight ratio)	80–90 nm	Johnson & Johnson	FDA (1995); EMA (1996, as Caelyx)	(77)
Myocet1	DOX	Liposome: phosphatidylcholine, cholesterol (55:45 molar ratio)	150nm	Teva	EMA (2000)	(78,79)
Abraxane1 (nab-PTX)	PTX	Human serum albumin NPs	130nm	Abraxis Bio-Science	FDA (2005); EMA (2009)	(80)
Mepact1	Mifamurtide	Liposome: POPC, OOPS	-	Takeda Pharmaceutical	EMA (2009)	(81)
Marqibo1	Vincristine sulfate	Liposome: sphingomyelin, cholesterol (58:42 molar ratio)	115nm	Talon Therapeutics	FDA (2012)	(82)
Onivyde1	Irinotecan	Liposome: DSPC, cholesterol, mPEG-DSPE (3:2:0.015 molar ratio)	110nm	Merrimack Pharmaceuticals	FDA (2015); EMA (2016)	(83)
Vyxeos J (CPX-351)	Cytarabine and daunorubicin (5:1 molar ratio)	Liposome: DSPC, DSPG, cholesterol (7:2:1 molar ratio)	100nm	Jazz Pharmaceuticals	FDA (2017); EMA (2018)	(84)
ApealeaJ	PTX	Micelle:twoisoformsof N-retinoyl-L-cysteic acid methylestersodiumsalt	20-30nm	Oasmia Pharmaceutical	EMA (2018)	(85)

6. Conclusion and Outlook

However, one of the biggest challenges in cancer chemotherapy is improving the tumor-specific targeting of nanopharmaceuticals. In this review, we summarized recent developments in CD44-targeted PLGA nanopharmaceuticals to enhance the therapeutic efficacy of the chemotherapeutic agents provided. However, some aspects should be taken into consideration when designing CD44 targeted PLGA nanopharmaceuticals. The preclinical results of the studies demonstrated superior efficacy of PLGA nanoformulations targeting CD44 compared to parent drugs. In vitro cell uptake studies performed using the inclusion of fluorescent dyes such as coumarin-6, FITC demonstrated NP receptor mediated internalization. It is also worth pointing out that CD44 expression varies between cell lines and therefore these criteria should be considered When designing experiments. Additionally, the developed nanoformulations have been shown in preclinical studies to alter the stability, toxicity, pharmacokinetic and pharmacodynamic outcomes of chemotherapeutic agents. However, most of the developed CD44-targeted PLGA nanoparticles have been studied using HA and therefore future perspectives should focus on comparing hyaluronic acid strategies with other targeting ligands used to targeting CD44. Furthermore, great importance should be placed on the mechanistic understanding of the complexity of CD44 variants and their interactions with targeting ligands in order to develop highly specific targeted therapies. It should also be noted that some of the delivery systems targeting CD44, such as irinotecan HA for the treatment of metastatic colorectal cancer (CRC), have reached clinical trials (73). Therefore, these criteria should be considered when preparing anti-CD44 nano-medicines.

Poly (lactic-co-glycolic acid) encapsulated platinum nanoparticles for cancer treatment

Table: 4 Summary of hyaluronic acid decorated PLGA nanoparticles for delivery of chemotherapeutic agents-

Chemotherapeutic agent	Method of preparation	Cell lines	Key outcome	Ref.
5-Fluorouracil	Nanoprecipitation	EAT	In vitro and in vivo studies demonstrated that HA conjugated NPs exert enhanced activity as compare to nontargeted NPs.	(63)
Doxorubicin	Dialysis	MCF7, MDA-MB-231	HA functionalized NPs enhanced both drug uptake and cytotoxicity in MDA-MB-231 cells as compare to MCF-7 cells.	(64)
	Dialysis	HCT-116	HA-g-PLGA micelle NPs demonstrated enhanced cellular uptake and higher cytotoxicity.	(65)
	Nanoprecipitation	EAT	In vivo antitumor efficacy studies demonstrated HA functionalized NPs delivered a higher amount of DOX as compare to un-functionalized NPs.	(67)
	Single emulsion solvent evaporation	HUVEC, 4T1	DCLK1–HA–PEG–PLGA NPs demonstrated targeting effect toward CSCs both in vitro and in vivo.	(69)
SN-38	Single emulsion solvent Evaporation	SKOV-3, OVCAR-8, CHO	In vitro cytotoxicity demonstrated enhanced cytotoxicity of PLGA-PEG-HA NPs in CD44 over-expressing cancer cells as compare to non targeted NPs.	(68)
	Emulsification	HGC27	Cellular uptake and in vivo biodistribution studies demonstrated the enhanced uptake and prolonged in vivo circulation of targeted NPs as compare to non targeted NPs.	(72)
Docetaxel	Dialysis	MCF7, MDA-MB-231	In vitro and in vivo efficacy studies demonstrated superior efficacy of HA NPs in CD44 over-expressing MDA-MB-231 cells as compare to native DTX.	(74)
	Nanoprecipitation	A549	HA coated NPs enhanced cytotoxicity and prolonged elimination half-life as compare to native drug.	(75)
	Nanoprecipitation	A549	DTX-rHP NPs exhibited enhanced antitumor activity towards CD44 overexpressing A549 lung cancer cells	(77)
Paclitaxel	Single emulsion solvent evaporation	MDA-MB-231	HA coated PLGA NPs enhanced the cellular uptake and cytotoxicity as compare to non-coated PLGA NPs.	(77)

Table: 4 Summary of hyaluronic acid decorated PLGA nanoparticles for delivery of chemotherapeutic agents-

Chemotherapeutic agent	Method of preparation	Cell lines	Key outcome	Ref.
	Nanoprecipitation	MCF-7, L929, U87MG	HA decorated PLGA NPs demonstrated superior in vitro and in vivo efficacy as compared to taxol.	(78)
Bromelain	Double emulsion solvent evaporation	EAC	HA functionalized NPs demonstrated enhanced cellular uptake and cytotoxicity is high CD44 expressing cells as compared to non-targeted NPs	(80)
TTQ	Single emulsion solvent evaporation	MCF7, MDA-MB-231, MiaPaca-2	TTQ loaded HA conjugated PLGA NPs significantly enhanced cellular uptake and cytotoxicity in CD44 overexpressing MiaPaca-2 cells.	(81)
ART	Solvent evaporation technique	SCC-7, MCF-7	ART loaded PLGA/HA NPs leads to significant reduction in cell viability as well as greater induction of apoptosis in CD44 over-expressing cancer cells.	(82)
Cisplatin	Double emulsion solvent evaporation	SKOV-3	HA conjugated PLGA nanoparticles demonstrated superior in vitro and in vivo efficacy as compared to cisplatin.	(83)
Doxorubicin,irinotecan	Double emulsion solvent evaporation	MDA-MB-231, PC-3	HA functionalized NPs demonstrated higher efficacy against both the prostasphere and mammosphere cells enriched with CSCs in vitro and CSCs in human breast tumor in vivo.	(84)
Docetaxel, tanespimycin	Single emulsion solvent evaporation	MCF7, MDA-MB-231, SCC-7 cells	In vitro cytotoxicity studies demonstrated that the combined use of DTX and 17-AAG at a molar ratio of 2:1 exhibited synergistic effect.	(84)
Salinomycin, Paclitaxel	Emulsion solvent diffusion	MCF7, MDA-MB-231	In vitro cytotoxicity studies demonstrated that combination of HA coated SLM NPs and PTX NPs were more potent than individual drug loaded NPs.	(84)
Doxorubicin, Cyclopamine	Double emulsion solvent evaporation	MCF7, MDA-MB-231	Dual drugs-loaded HA-SS-PLGA NPs demonstrated enhanced inhibition to the tumor sphere formation as compare with drug-loaded PLGA particles.	(81)

Table: 4 Summary of hyaluronic acid decorated PLGA nanoparticles for delivery of chemotherapeutic agents-

Chemotherapeutic agent	Method of preparation	Cell lines	Key outcome	Ref.
Camptothecin, Curcumin	Single emulsion solvent evaporation	Colon-26	Cellular uptake experiments demonstrated HA decorated NPs enhanced cellular uptake in colon cancer cell line as compare to un-targeted NPs.	(82)
GA, TRAIL plasmid	Dialysis	MCF-7, MDA-MB-231, 4T1	Dual drugs-loaded NPs significantly enhanced apoptotic cell death in vitro and inhibited TNBC tumor growth in vivo.	(85)

DISCUSSION

These results are consistent with other published studies showing anticancer activity of Pt-NPs1, 20 and no toxicity in healthy cells.10,19 To increase cellular uptake of Pt-NPs, a targeted PLGA particle delivery system was used.

To achieve passive and active targeting of the particles, surface modifications were performed, producing two different sets of particles: Pt-PLGA-PEG (PEGylated) and Pt-PLGA-PEG-EGFR (conjugated with EGFR antibody).

Cellular experiments were performed with both sets of particles against TNBC and showed more cell death with Pt-PLGA-PEG-EGFR than with Pt-PLGA-PEG, confirming cell targeting by the antibody.

When comparing Pt-PLGA-PEG-EGFR particles with free PtNPs, a more potent anticancer effect of free PtNPs was observed, possibly due to the time required to release PtNPs from PLGA for Pt-PLGA-PEG-EGFR particles

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Conflict to Interest

There is no any conflict of interest by authors.

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Ethical Declaration

Since the study is in compilation form, it does not require ethics committee approval.

Authorship Contributions

Concept: SJ, RK, AR, Design: SJ, RK , AR ,Supervising: SJ, RK, AR, Financing and equipment: SJ, RK, AR, Data collection and entry: SJ, RK, AR, Analysis and interpretation: SJ, RK, AR, Literature search: SJ, RK, AR, Writing: SJ, RK, AR, Critical review: SJ, RK, AR.

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