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Review Article

RECENT ADVANCES IN NANOCARRIER BASED THERAPEUTIC AND DIAGNOSTIC TOOLS FOR COLORECTAL CANCER

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ABSTRACT

Colorectal cancer (CRC) is among the frequently diagnosed cancers and one of the leading causes of deaths in the world. It has remarkably high rates of metastasis, which incidentally is one of the leading causes of CRC related deaths. Ineffective drug concentration at the desired site of action and toxicity due to peripheral targeting limits the efficacy of the conventional chemotherapeutic treatments. Currently used traditional diagnostic tools have various shortcomings such as poor intracellular contrast between malignant and benign cells and low detection sensitivity in biological environment. Smarter drug delivery systems based on nano carriers have been proven remarkably promising in enhancing drug distribution and bioavailability, increasing half-life and achieving targeted drug delivery, thus, minimizing toxicity. Diagnosis employing nanoparticles is more effective in terms of stability, duration and efficiency. CRC targeting, both for drug delivery as well as diagnosis, is improved manifold by incorporating ligands of tumor specific surface receptors on the nanoparticles. Recently documented data have furnished cogent evidence apropos, the potential of active-targeted nanotherapeutics, and diagnostics in CRC therapy involving myriad forms of nanoparticles. This review deliberates the current status of nanocarriers, and the significance of their use in colorectal cancer therapy.

Keywords: Colorectal cancer, Nanocarriers, PEGylated liposomes, Chitosan nanoparticles, Gold nanoparticles, Diagnostic tools, Targeting ligands.

INTRODUCTION

As per GLOBOCAN 2012, colorectal cancer (CRC) is one of the major causes of cancer related deaths worldwide with nearly 1.4 million cases diagnosed globally [1]. It is the third most common cancer in men and second most common cancer in women (10% and 9.2% of the total cases worldwide respectively for men and women) [1]. The strategy for the prognosis and treatment of CRC depends upon the stage of the disease. But the key to an efficient prognosis of a disease lies in its efficient diagnosis. Existing diagnostic tools for the diagnosis of CRC is efficient, but they also have a number of shortcomings. Invasive techniques like sigmoidoscopy, colonoscopy, incisional biopsies, and barium enemas involve discomfort to the patient [2-5]. Most examinations are also not able to examine the entire colon. Furthermore, they cannot detect early neoplasia, or short lesions [2, 3]. Diagnosis from these techniques depends on the knowledge. Skill and judgment of the physician, and is time consuming [2-5]. Imaging techniques employ organic dyes and radioactive compounds. They have deficiencies such as poor hydrophilicity and photostability, low quantum yield along with insufficient stability in biological system, and low detection sensitivity all leading to low efficiency [6]. The use of radioactive compounds also has significant biological risk. Therefore, new improved diagnostic tools for the detection of CRC are vital. The treatment paradigm for CRC generally involves surgery, along with some adjunct chemotherapy as the primary approach of treatment if the cancer is in the early stages. However, roughly 25% of patients with CRC develop overt metastatic disease; also 40 to 50% of the newly diagnosed patients develop metastasis [7]. Surgery is often carried out with curative intent for late stage metastatic CRC, but, over one half of the patients develop recurrence within 2 years [8]. The primary site of CRC metastasis is the liver. At the time of diagnosis approximately 20% of patients have existing synchronous liver metastasis, and the other 30% of patients develop liver metastasis after resection of the primary CRC [9]. Hence, for late stage CRC, chemotherapy is the main line of treatment. The conventional chemotherapy for CRC includes drugs such as, 5-fluorouracil along with leucovorin, irinotecan, oxaliplatin and combined drug therapies like 5FU/lV or 5FU/lV/oxaliplatin (FOLFOX) and a combination of 5FU/FA/irinotecan (FOLFIRI) [10]. Apart from drugs, biotechnological products such as monoclonal antibodies like Cetuximab are being considered as the first line treatment for CRC [7]. Conventional therapy has its own limitations, like low circulation time, lack of site specificity, rapid degradation, high dosage, all leading to undesirable side effects, and toxicity. Recently, there has been a surge in the field of nanotechnology and nanomedicine that has given an impetus to sustained and site specific drug delivery by using nano-sized drug delivery systems. These nano-sized carriers of drug also have the capacity to carry high payloads [11]. They can also be tailored by attaching polyethylene glycol (PEG) chains on to the surface of the nanocarriers to evade the Reticulo-endothelial system (RES) to have long circulation time in the blood stream [12, 13]. The physiology of the leaky tumor vasculature is also favorable to the nanocarriers, allowing them to accumulate in the tumors through the enhanced permeability and retention effect (EPR) [14]. These nano sized carriers have the advantage of having favorable surface characteristics that enable them to be functionalized to target the desired site of action [3]. Lower accumulation rates in healthy tissues, coupled with higher accumulation and retention rates in tumor tissues, elucidate the higher efficacy, and minimal side-effects [12]. The pliable surface property of the nanocarriers is also advantageous for cancer diagnostics, because they allow the development of new enhanced techniques for specificity in molecular imaging [15]. Hence, nanocarrier based diagnosis, therapy as well as the ranostics is the answer to all the woes of the available conventional options.

Targets for colorectal cancer

The advancement in the field of molecular biology has been burgeoning, leading to the discovery of newer molecular targets for CRC. This advancement stems from the detailed understanding of the intrinsic molecular mechanisms of carcinogenesis [16]. Folate receptors (FRs) are over expressed on the endothelium of many cancer cells. They are especially over expressed on the surface of CRC cells [17, 18]. FRs are tumor specific since they become available to drugs in the systemic circulation only after malignant transformation of the cell [17]. Folic acid (FA) is the ligand that has high affinity for FRs. It is a stable molecule, easy to process, and is less expensive [19, 20]. Accordingly, site specific delivery to CRC cells can be attained by attaching the nanocarrier with FA. Efficient internalization via FA conjugated nanocarriers can be ensured as FA is one of the essential nutrients of a cell [20, 21]. Hyluronic acid (HA) is a molecule which is present in the extra, and peri cellular matrixes

[22]. It plays a significant role in the basic cellular activities such as cell adhesion, cell migration, cell-cell recognition, and cell differentiation [22]. CD44 is the receptor for HA. Over and co-expression of epithelial HA and CD44v6 (the splicing variant of CD44) enhances tumor progression and metastasis [23]. Nanocarriers conjugated with HA can achieve targeted delivery to the CD44 receptors [24]. In this way, the delivery of drugs or diagnostic tools can be accomplished even to the regions where the tumor has metastasized. Carcinoembryonic antigen (CEA) is a membrane-bound glycoprotein that is expressed in over 80% of colorectal cancers with relatively less expression in normal mucosa [25, 28]. CEA was found to have the best sensitivity (93.7%) and specificity (96.1%) for CRC detection [25]. CEA as a viable target, thus, has a great potential in the prognosis of CRC [26-28]. Latest studies illustrate the role of chemokines and their receptors in organ selective metastasis [29, 32]. Specific chemokines are produced and released by target organs to attract tumor cells with specific corresponding receptors [29]. This results in site and/or organ-specific cancer cell migration and metastasis [29]. CRC shows organ specific migration to the liver. Among the chemokines, CXCR4 has the major role in the metastasis of CRC. If CXCR4 is nullified, there will be a substantial inhibition of Tumor metastasis [30-32]. Angiogenesis, the development of new blood vessels to supply nutrients, oxygen, and growth factors is essential for tumor growth and metastasis [33]. Expression of several integrin molecules like α5β1, α1β3, α4β1 which are not expressed in normal endothelial cells is a hallmark of cancer [35]. Integrin α5β1 is especially up regulated on CRC cells [34]. Chemoprevention has lately captured the attention of the world, and is now an emerging science. It is essentially a preventive therapy which involves the use of agents that will prevent, inhibit, delay, or reverse the carcinogenesis in CRC [36]. A target that is gaining importance in this newly emerging field is cyclooxygenase-2 (COX-2). The involvement of COX-2 in the promotion of CRC is being studied largely [37]. Chronic use of Non-steroidal anti inflammatory drugs (NSAIDs) has shown to reduce the risk of CRC in humans by 40-50% [38]. Studies have indicated that 85% of the primary CRC cells over express COX-2 [38]. The *in vitro* and *in vivo* studies have demonstrated that inhibition of COX-2, leads to inhibition of tumor growth and development. Selective COX-2 inhibitors have been reported to reduce the formation, growth, and metastasis of experimental tumors [39]. Most importantly, pre-clinical studies have recently exhibited strong anti-cancer effect of selective COX-2 inhibitors against CRC [40]. Current findings regarding the mechanism governing COX-2 inhibitors show that anti-cancer activity against CRC suggest cell cycle arrest at G0/G1 phase [37], apoptosis, [36] and inhibition of angiogenesis [36]. Epidermal growth factor receptor (EGFR) is a transmembrane receptor. The EGFR gene is over expressed, or up-regulated in 60–80% of colorectal cancers, thus making it a suitable candidate as a molecular target for targeting CRC cells [41].

Nanocarriers as a diagnostic tool for colorectal cancer

The failure of CRC detection leads to the progression of the disease, and its subsequent metastasis to other organs [24]. The development of materials in the nano size range for application in biomedical imaging technique has gained a lot of attention lately. Nanocarriers such as gold nanoparticles (AuNPs) [3, 15], hyaluronic acid nanoparticles (HA-NPs) [24], superparamagnetic iron oxide nanoparticles (SPIONs) [26] and magneto-fluoro silica nanoparticles [42] have been used for aiding imaging techniques for the detection of CRC. AuNPs has a size-tunable surface plasmonresonance. This leads to strong absorption, and scattering in the visible-to-near-infrared region [15]. This feature gives the AuNPs an edge above the conventional dyes that are used for biomedical imaging. AuNPs has the capacity to get attached readily to amines, thiols, and disulfides. Accordingly, they can be modified by attaching a number of targeting ligands such as proteins, DNA, peptides, etc. As they are metallic nanoparticles, they do not suffer from the common demerits of organic dyes such as photobleaching [15]. They also seem to be biocompatible. Unlike radioactive compounds AuNPs also are not a biohazard. In a study conducted by K. M. G. Lima *et al.*, AuNPs were conjugated with antibodies anti-$\alpha_5\beta$ catenin, and anti-E-cadherin to specifically target CRC cells as these are over expressed on CRC cells [15]. The authors demonstrated that the confocal images using their technique were at par with the standard technique which uses Alexa Fluor®488 (an antibody conjugated with a dye). The procedure put forth by the authors was also faster taking only 1hr as opposed to 27 hrs using Alexa Fluor®488 [15]. As these targets are hallmarks of metastasized CRC, effective and fast imaging technique targeting the same will be an enormous breakthrough for CRC diagnosis. AuNPs are also used for a non-invasive diagnostic technique for determining blood serum biochemical information using surface enhanced Raman spectroscopy [3]. They help overcome the limitation of Raman spectroscopy which when used as such gives poor signals. Surface enhanced Raman spectroscopy has the advantage of differentiating between healthy and cancerous cells with a diagnostic sensitivity of 97.4% and specificity of 100% using the principal composite analysis-linear discriminant analysis [3]. AuNPs are also preferred over commonly used silver, due to their physical and chemical properties and biocompatibility [42]. The biochemical target HA can be used as a the ranostic agent, i.e. it can be used to diagnose as well as treat at the same time [24]. K. Y. Choi *et al.* outlined this in their research where they prepared the polyethylene glycol conjugated hyaluronic acid nanoparticles (P-HA-NPs) [24]. These P-HA-NPs selectively accumulate in tumor tissues which over express HA receptor CD44 or CD44v6. The authors indicated that for diagnostic purposes, a near-infrared fluorescence imaging dye (Cy 5.5) was chemically conjugated onto the HA backbone of P-HA-NPs. After intravenous injection of Cy5.5-P-HA-NPs into the Azoxymethane induced orthotropic tumor-bearing mice, small-sized colon tumors (HT29 cells) as well as liver-implanted (CT26) colon tumors could be visualized efficiently using the near-infrared fluorescence imaging technique [24]. Inorganic magnetic nanoparticles have customarily been exploited as a contrast media for molecular imaging, because of their magnetic properties [43]. T. J. Yoon *et al.* synthesized silica-coated and organic dye-incorporated iron oxide nanoparticles (MFSN) that allowed the detection of fluorescence in cells and tissues and characterization of magnetic properties by the use of magnetic resonance imaging (MRI) [44]. Silica coating of MFSN reduced the cytotoxic effects of the particles resulting from direct exposure to heavy metals. Furthermore, it prevents the photobleaching of the fluorescent dye [44]. Cetuximab (Ctx) is an immunoglobulin G1 mouse–human chimeric monoclonal antibody. It has high affinity and specificity towards the human epidermal growth factor receptor [45]. Y. S. Cho *et al.* carried out a study in which the properties of both the MFSN and Ctx were exploited. MFSN-Ctx could specifically target colon cancer cells that express EGFR on their cell membrane [46]. As a result, discernable fluorescence signals could be produced, and incite magnetic resonance signal alterations *in vivo.* These signals were not produced when plain MFSNs were used [46]. The nanoparticles were completely evacuated from tumor after the diagnosis. Correspondingly, MFSN-Ctx enabled a noninvasive as well as a non-toxic imaging and quantification technique for EGFR expression *in vivo*, and may provide a clinically transferable strategy to diagnose CRC [46].

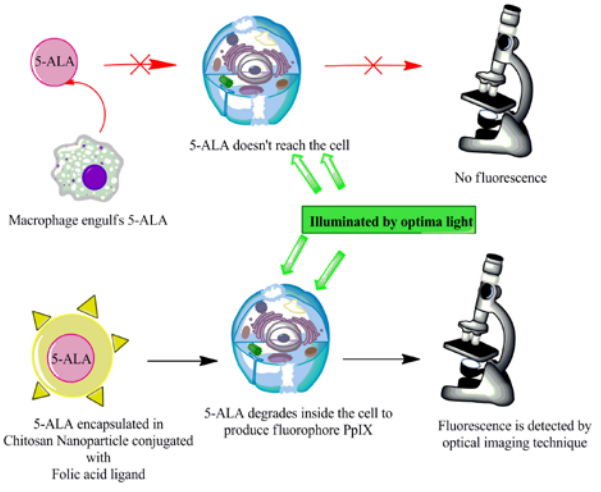

Fig. 1: Mechanism of delivery of 5-ALA using FA conjugated Chitosan NP for diagnosis of CRC

As described in the targets for CRC section, CEA is a membrane bound glycoprotein that is over expressed in CRC cells. It also has high specificity (100%) for colorectal cancer detection [25, 28]. Anti-CEA-functionalized SPIONPs were synthesized in a study by Huang *et al.* for labeling colorectal tumors by conducting different pre-operative and intra-operative *in vivo* examinations using MRI, and scanning superconducting-quantum-interference-device bio susceptometry (SSB) respectively in CT26 induced tumors in mice [26]. The results were verified using ICP to prove that the results obtained from MRI and SSB were indeed from the tumor cells [26]. Optical imaging by MRI commonly uses gadolinium. There is conclusive proof that gadolinium induces kidney disease [47]. SPIONPs pose a lower threat than other superparamagnetic substances. Consequently, SPIONPs labelled with bioprobes have been developed for highly specific targeting of tumors [48, 49]. CEA targeted Anti-CEA conjugated Dye-doped silica nanoparticles were also developed which show live, *in vivo* fluorescent imaging in a murine model of colorectal cancer [27]. This technique holds promise for clinical translation in the context of intra-operative imaging, since fluorescence obtained is bright, the antibody is humanized, and silica nanoparticles appear to have favorable toxicity profiles [27]. An exogenous chromophore, such as protoporphyrin IX (PpIX) is used to generate fluorescence in cancer cells by exciting it by optima light [50, 51]. PpIX is formed when its precursor 5-aminolevulinic acid (5-ALA) is totally degraded inter cellularly. The decomposition rate of the photosensitive fluorophore PpIX in cancer cells is different compared to the rate in normal cells. Hence, it can be used to differentiate between cancer cells and healthy cells. However, the major drawback of using 5-ALA is that it is almost immediately engulfed by the bacteria in the gastrointestinal tract. Yang *et al.* fabricated a folic acid conjugated chitosan nanoparticle (fCNA) as a vehicle for carrying 5-aminolaevulinic acid (5-ALA) with a loading efficiency of 35 to 40% and a size of 100 nm. HT29 and Caco-2 colorectal cancer cell lines were utilized to ascertain the rate of accumulation of protoporphyrin IX (PpIX) by using fCNA. As these cell lines over express folate receptor on the surface of their cell membrane, f CNA were uptaken in a short time via receptor mediated endocytosis. Thus, it can be inferred that fCNA can be used as an ideal carrier for the detection of CRC [50].

Nanocarriers for targeted drug delivery to colorectal cancer cells

Lipid based drug delivery

Liposomes are sphere shaped vesicles consisting of one or more layers of phospholipids. [12]. They are prepared using phospholipids, cholesterols, non-toxic surfactants, sphingolipids, glycolipids, long chain fatty acids, and even membrane proteins. Liposomal drug delivery is characterized by slow and delayed release, passive targeting by EPR effect and high drug loading leading to reduction in dose [12]. Active targeting can be achieved by modifying the surface of the liposomes. The first nanocarrier based formulations to get FDA approval are the liposomal anticancer formulations [52]. Extensive research has been conducted over the years and is being carried out in the field of lipid based drug delivery for the treatment of CRC. Cytotoxic drugs are non-selective between normal and tumor tissue. This poses a challenge to the strategy devised for the treatment of tumors. Liposomes for systemic applications can be designed to be small (100–200 nm) so that they can escape their uptake by the phagocytes [53]. Long circulation time can be attained by attaching chains of PEG to the surface of the liposomes [53]. The PEG chains provide steric stabilization to the liposomes, as a result the lipsomes are protected from getting opsonized and are not removed from the blood circulation by the RES. Oxaliplatin (trans-l-diaminocyclohexane oxalatoplatinum, (L-OHP) is a third-generation platinum analogue with proven anti-tumor activity against many tumor cell lines [54]. However, it does not show sufficient anti-tumor activity *in vivo* when used alone [55, 56]. In order to overcome this problem, L-OHP was encapsulated into PEG-coated cationic liposomes [54] and long-circulating liposomes (PEG-liposomal L-OHP) [57]. The effects of PEG-liposomal L-OHP in human colorectal carcinoma cell line (SW480) induced tumor in female BALB/c nude mice were studied. PEG-liposomal L-OHP brought about notable apoptotic response as compared to the free L-OHP, 23.21%±3.38% vs. 16.85%±0.98%, respectively [57]. *In-vivo* fluorescence imaging showed that PEG-liposomes specifically targeted tumour tissue. After intravenous injections of PEG liposomal L-OHP or free L-OHP, the tumour volume suppression ratio was 26.08%±12.43% and 18.19%±7.09%, respectively, the percentage increase in the life span (ILS%) was 45.36% and 76.19%, respectively, and Bcl-2, Bax mRNA and protein expression in tumour tissue was 0.27-fold vs. 0.88-fold and 1.32-fold vs. 1.61-fold compared with free L-OHP, respectively [57].

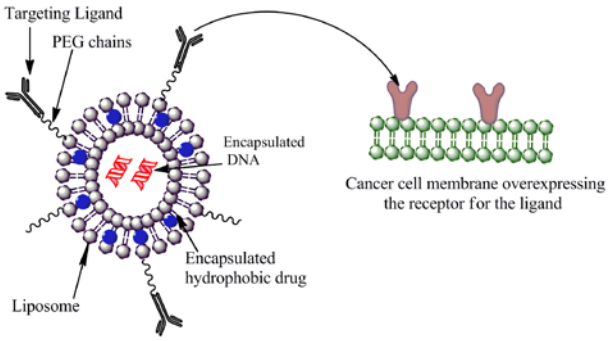

Fig. 2: Targeted drug delivery by Lipid based drug delivery system

A single injection of L-OHP encapsulated in PEG-coated cationic liposomes achieved complete suppression of angiogenesis in the (Dorsal air sac) DAS assay, while injection of either free L-OHP or L-OHP encapsulated in PEG-coated neutral liposomes only very slightly suppressed angiogenesis [54]. Integrin $\alpha_5\beta_1$ is expressed on several types of cancer cells, including colon cancer and plays a critical role in tumor growth and metastasis. Researchers have utilized RGD peptide-based techniques to target the integrin $\alpha_5\beta_1$, but as it has several targets, achieving specificity is a big challenge. A. Garg *et al.* have developed a novel peptide-amphiphile sequence referred to as PR_b that closely mimics the cell adhesion domain of fibronectin. PR_b peptide-amphiphile was encapsulated into stealth liposomes with the goal of targeting integrin $\alpha_5\beta_1$ that is expressed on colon cancer cells [58]. Its efficacy was studied on CT26. WT, RKO, HCT116 cell lines. It was proven that PR_b peptide-amphiphile encapsulated stealth liposomes had better efficacy than RGD peptide. This sytem was found to be as effective as 5-FU [60]. PEG-liposomes (PEGylated liposomes) are also efficient in gene delivery. Branched polyethyleneimine (bPEI) condensed plasmid DNA was encapsulated into targeted stealth liposomes. PR_b was used to functionalise the liposome and target integrin $\alpha_5\beta_1$. Targeted PEGylated liposomes outperformed non-targeted and non-encapsulated gene showing better transfection [59]. 5-Fluorocytosine (5-FC) is used in gene-directed enzyme pro drug therapy entailing Escherichia coli cytosine deaminase. The delivery of drugs to tumors is generally low, due to their insufficient transfer from the blood. Tumor specific promoters and virus based vectors were used to enhance the activity of 5-FC rather than considering options to deliver it in an efficient way to the solid tumors. To overcome this deficiency the pro drug 5-FC, was encapsulated in liposomes composed of di palmitoyl phosphatidyl choline (DPPC) and cholesterol (1:1). When the liposomal form of 5-FC was administered intravenously., MC38/7 tumor bearing mice they showed complete regression of transplanted tumors and complete cure was observed, whereas in animals treated with free pro drug, 50% tumor regression, and only insignificantly prolonged median survival were found [60]. Cationic liposomes complexed with recombinant adenoviral vectors have also proven useful in Coxsackievirus deficient cells to enhance therapeutic gene transfer *in vivo* to murine CT26 tumor model. This is another example of gene delivery to CRC murine model [61]. Celecoxib (CLX) is a hydrophobic COX-2 inhibitor with proven activity against colorectal polyps [53]. However, its adverse cardiovascular side effects restrict its use. Liposomes are carriers which can encapsulate hydrophobic

drugs in their hydrophobic bilayer. Liposomal formulation was fabricated using 1,2-Distearoyl-sn-glycero-3-phosphocholine, cholesterol, and polyethylene glycol [53]. Flow cytometry and confocal microscopy studies were used to study the cellular association, and internalization using the colon cancer cell lines HCT-116 and SW620. The results establish that the cell lines showed significantly higher cellular association and internalization of the liposomes as the function of time. Cell proliferation was inhibited by 95% and 78%, respectively, in SW620 and HT29 cells after incubation with 600 µl liposomal CLX for 72 hrs [53]. Apart from carrying drugs, liposomes have also been studied for the delivery of magnetic nanoparticles. Citric acid-coated magnetic nanoparticles (CAMNP) and doxorubicin were encapsulated into the liposome made of HSPC, DSPE and cholesterol. CAMNP were found to be more efficacious than conventional Doxorubicin liposome [62]. Hybrid liposomes (HLs) are modified liposome wherein the vesicles are sonicated with micellar solutions of surfactants. HL composed of L α dimyristoylphosphatidylcholine(DMPC) and polyoxyethylene(*n*) dodecyl ethers (C12(EO) n, n = 21, 23, 25) were fabricated using sonication method. The inhibitory effects of HL were investigated on human colon cancer HCT116 cells *in vitro*. The formulation was effective and acted by inhibiting cell cycle arrest at G_0/G_1 phase. The hybrid liposomes could also differentiate between healthy and tumor cells [63]. Hybrid liposomes also show remarkable effects on Xenograft model in mice [64]. Solid lipid nanoparticles are another form of lipid based drug delivery system made up of solid lipids and surfactants. Butyric acid is a short-chain fatty acid found naturally in the colon. It influences various physiological processes like cell growth and apoptosis in CRC cells [65]. Thus, chol-butyrate solid lipid nanoparticles (SLNs) were formulated, and tested on the colon cancer cell line HT-29. It showed anti-proliferative activity of around 50% growth inhibition of the cells at 0.3 mM concentration [66]. Natural polyphenols such as flavanoids are extensively distributed throughout the plant kingdom. Quercetin is a flavanoid which is slightly soluble in water, but it exhibits a variety of biological and pharmacological activities, anti-cancer activity, and anti-inflammatory effects to name a few [67]. It has been proved recently that QT can arrest the proliferation of colon cancer [68]. However, it has minimal gastrointestinal absorption, and low bioavailability. The answer to this would be to encapsulate QT with SLNs. Upon oral administration in rats, the bioavailability of the QT-SLNs suspension was much higher (more than 5 times) than free QT. This proves that SLNs are favorable carriers for poorly water-soluble drugs such as QT [69].

Chitosan based nanocarriers for targeted drug delivery to colorectal cancer cells

The primary site to which metastasis occurs in CRC is the liver. And 70% of the CRC related deaths occur due to liver metastasis [70]. Interleukin-12 (IL-12) is a cytokine that reinforces cellular immunity, T helper cell 1 (Th1) differentiation, proliferation of natural killer, and activated T cells [71]. It has been manifested to be one of the most effective inducers of strong antitumor immunity, but it has the disadvantage of excessive toxicity on intravenous administration [72]. Q. Xu *et al.* utilized chitosan (CS) nanoparticles as carriers for interluekin-12 (IL-12) by incorporating IL-12 in chitosan using tripolyphosphate (TPP) as the coacervated crosslinking agent to form CS-TPP/IL-12 nanoparticles [73]. Systemic delivery of CS-TPP/IL-12 nanoparticles significantly reduced the number, and volume of CRC liver metastasis as compared to the CS-TPP treated mouse group. [73]. CS nanoparticles can also be used to ameliorate the effects of CLX [74] which is the only coxib that is approved for adjuvant treatment of patients with familial adenomatous polyposis [75]. But because of its wide tissue distribution and 97% plasma protein binding, the oral dose recommended for CLX is high, which in turn escalates the concerns about serious possible side effects. Chitosan hydroxyapatite nanocomposite mediated delivery of celecoxib represents a feasible strategy to reduce the side effects associated with celecoxib. Also, hydroxyapatite has been reported to produce cytotoxicity against sarcomas and carcinomas, particularly colorectal carcinomas [76]. Celecoxib-loaded Hap-CS nanoparticles showed significant antiproliferation, apoptosis and time-dependent cytoplasmic uptake in HCT15 and HT29 cell lines tumor regression on xenograft model in mice [74]. As another conclusive proof that nanoparticles has favorable surface properties, P. Li *et al.* conjugated CS with FA by chemically linking carboxylic group of FA with amino group of CS. Cellular uptake studies by fluorescent microscopy using calcein as fluorescent marker in colon cancer cells (HT-29) exhibited enhanced uptake of FA conjugated CS nanoparticles in HT-29 [77]. Efforts for colon targeting resulted in the design of Eudragit S100–coated pellets encapsulating HA–coupled CS nanoparticles bearing oxaliplatin (L-OHP). These targeted nanoparticles were preferentially delivered to the colon as Eudragit S-100 is an entero protective agent [78]. The bio distribution studies indicated that HA-coupled nanoparticles reached the tumor, and proved to be more efficacious in treating colon tumors as compared to uncoupled CS nanoparticles, and the free drug as HA is overexpressed on CRC cells [78]. The oral route of administration is the most patient compliant. A. M. Urbanska *et al.* made efforts to make oral delivery of Oxaliplatin conceivable by using a novel 'particle in particle' technique. The authors first loaded oxaliplatin into lipid like polymeric nanoparticles which were further encapsulated in mucoadhesive micro-sized alginate based particles. They were evaluated *in vivo* and the results depicted an increased survival and decreased tumor progression after 17 weeks of treatment in comparison with the control group. These NPs also led to a lower serum concentration of proinflammatory cytokines (IL-12, IL-6), lactoferrin, and C-reactive protein (CRP) [79].

Other nanocarriers for targeted drug delivery to colorectal cancer cells

Recent discoveries of newer molecular targets have led to the better targeting options. Nanoparticles conjugated with novel ligands can be used to target the molecular targets. Thus achieving enhanced site specific drug delivery. Pectin is a family of complex polysaccharides, found in large quantities in plant wall. Pectin and its derivatives like modified citrus pectin have proven anti-cancer activity. Modified citrus pectins act by impairing cell-cell interaction by binding with galectin-3 receptors (galactoside binding site). Galectin-3 receptors are over expressed on colorectal cancer cells [80]. Citrus Pectin Nanoparticles (E-CPNs) of 5-FU were used to specifically deliver 5-FU to Galectin-3 expressing CRC cells. These CPNs were further coated with Eudragit S 100 to ensure efficient delivery to the colon. Sulphorhodamine B assay to determine the *in vitro* cytotoxicity against HT-29 cancer cells exhibited 1.5 fold greater cytotoxicity potential of nanoparticles as compared to free 5-FU solution. *In vivo* data depicted that the nanoparticles guarded by Eudragit S100 successfully reached the colonic region wherein the nanoparticles were taken up, and showed drug release for an extended period of time [81]. Literature evidence suggests that a lot studies have proven that Bufalin (Dried toad venom from *Bufo gargarizans*) has significant antitumor effect in a variety of tumors. Shortcomings due to toxicity, water insolubility, rapid metabolism and short half-life limit its application in cancer therapy. To overcome these deficiencies, Hu *et al.* explored the anti-metastatic role of Bufalin-loaded pluronic polyetherimide nanoparticles on HCT116 colon cancer-bearing mice [82]. The *in vitro* release data showed that free bufalin was released faster compared to the bufalin-loaded pluronic polyetherimide nanoparticles, and almost 80% of free bufalin was released after 32 hours. Results showed that nanocarriers based on pluronic PEI nanomaterials with controlled release action protected normal tissues against harm from Bufalin during blood circulation [82]. Antiangiogenic therapy using Vincristin is a validated treatment approach for CRC. What hinder effective therapy using Vincristin (VCR) are the adverse effects due to off target delivery. Therapy based of nanoparticles uses the EPR effect for tumor localization of drugs. In certain tumors which have less vascularization and multiple small metastases, EPR is greatly compromised. For such tumors targeting the tumor vascular endothelial cells using targeted nanoparticles is a feasible option [83]. C. Wang *et al.* studied the nanoparticles made up of block copolymers encapsulating VCR conjugated to F56 peptide targeting the vascular endothelial growth factor-1 (VEGRF-1) receptors. VEGFR-1 is the vascular endothelial growth factor receptors which are over expressed on tumor vasculature. The F56-VCR-NP could extend the half life of VCR from 2.98 h to 32.08 h. The tissue distribution study also showed that DiR dye labeled F56-NPs localized more in the HCT15 xenograft in mice than plain NPs

labeled with DiR dye. After 2 hrs of administration the coumarin 6-labeled F56-NP y targeted the vessels of both subcutaneous HCT-15 tumors and the CT-26 lung metastases in mice. While the non-targeted NPs extravasated and spread extensively in the tumor parenchyma through the EPR effect. F56-VCR-NP induced extensive tumor necrosis of about 45.4% compared to 16.1% by VCR-NP. This study proved the significance of nanoparticle based targeted antiangiogenic therapy to the tumor vascular endothelial cells and that it shows promising results for the therapy of CRC and its lung metastasis [83]. As described in the targets for CRC section, CXCR4 is expressed on cancer cells. CXCR4 over expression leads to organ specific metastasis [30-32]. RNA silencing is a technology which is being encouraged greatly for the treatment of various cancers [32]. RNA silencing is triggered by siRNA and the delivery of siRNA to organs holds the answer for the treatment of diseases such as cancer. The delivery of siRNA however poses difficulties such as instability in the blood and lack of delivery options [32]. Abedini *et al.* carried out a study to develop a vehicle for delivering CXCR4 siRNA. The authors demonstrated that dextran-spermine nanoparticles could successfully deliver CXCR4 siRNA. Dextran-spermine is a novel cationic biodegradable polymer which has been used for gene delivery. The study illustrated that the control group which received control siRNA showed highest CXCR4 expression (8.09±0.8 fold) and the group which received CXCR4 siRNA dextran-spermine showed lowest CXCR4 expression (2.49±0.04 fold). The lower expression of CXCR4 proved that dextran-spermine effectively delivered the siRNA which silenced the RNA responsible for the expression of CXCR4 protein [32].

Table 1: Recent patents and patent applications in the field of nanocarrier based diagnostic tool and drug delivery for CRC

Patent No./Patent Application No.	Assignee	Description	Reference
US/8,197,471 B1	Samuel Harry Tersigni, Alpha, NJ (US)	Core-excited nanoparticle thermotherapy (CENT) bound to targeting agents that deliver them tumor cell.	[85]
US 2011/0111044 A1	Enzon Pharmaceuticals INC., BridgeWater, NJ (US)	Nanoparticle compositions for the delivery of oligonucleotides encapsulated inamixture of a cationic lipid, a fusogenic lipid and a PEG lipid.	[86]
US 2009/0169478 A1	Board of Supervisors Of Louisiana State University, Baton Rouge, LA(US)	Magnetic nanoparticles (MNPs) that carry a therapeutic agent and ligands with a specific target cell receptor.	[87]

Nanocarrier can also be used as a platform for simultaneously delivering two drugs with different

Table 2: Recent clinical trials in nanocarrier based therapy of CRC

Drug	Delivery system	Phase	Sponsorer, Organisation	Clinical Trials Gov. identifier	Description	Status	Reference
Gemcitabine plus nab-Paclitaxel	Albumin Nanoparticle	I	Plexxikon	NCT01804530	Evaluation of PLX7486 alone on patients with advanced non-recectable tumors.	Recruiting	[88]
CRLX101 (camptothecin)	Nanoparticle	I & II	UNC Lineberger Comprehensive Cancer Centre, Cerulean Pharma Inc.	NCT02010567	Camptothecin NPs gave along with Capecitabine as well as radiation therapy.	Recruiting	[89]
Paclitaxel	Nanoparticle	I	CritiTech, Inc., University of Kansas Medical Center Research Institute, Inc. Beckloff Associates, Inc.	NCT00666991	Evaluation of pharmacokinetics, safety and efficacy of the formulation.	Completed	[90]
TKM-080301	Lipid Nanoparticle	I	National Cancer Institute, National Institutes of Health Clinical Center (CC)	NCT01437007	Evaluation of the efficacy of the new drug for cancers like CRC that has metastatised to the liver which do not respond to other drugs.	Completed	[91]

Table 3: Recent clinical trials in nanocarrier based diagnosis of CRC

Drug	Delivery system	Phase	Sponsorer, Organisation	Clinical Trials Gov. identifier	Description	Status	Reference
Diagnostic Tool	Nanoparticle	Unknown	Rambam Health Care Campus, Technion, Israel Institute of Technology	NCT01292369	Nanopaticle based system for diagnosis of Colon cancer by breath test by analyzing the volatile oil compounds in the breath which is high patients with cancer due to lipid peroxidation in cell membranes due to stress.	Unknown	[92]

Modes of action in a single carrier for illustrating synergistic action. A biodegradable polymer mPEG-PLA (methoxyl-polyethylen glycol-block-polylactide) was first conjugated with gemcitabine. Gemcitabine is a drug which shows anticancer activity by inhibiting DNA synthesis. This drug conjugate was then used to encapsulate curcumine, a versatile molecule obtained from *Curcuma longa*. The final polymeric micelles could overcome the deficiency of low solubility and low bioavailability of curcumine and gemcitabine respectively. The M (Cur/Gem) showed better synergy *in vitro* in HCT-116 cells. It also showed better anti tumor activity *in vivo* in nude mice bearing HCT-116 cells xenograft [84].

Recent patents and clinical trials

The application of nanotechnology in the field of colon cancer diagnosis and treatment has led to frequent patenting of the technology. Due to the immense potential in this field, a lot of research is being translated into clinical trials. A summary of the recent patents and clinical trials in the field of nanocarrier based diagnostics and therapeutics for CRC is given in table no.1 and 2.

CONCLUSION

Nanoparticles as carriers of drugs and diagnostic tools to cancer cells have enhanced their efficiency to a great extent. They have improved the bioavailability of anti-cancer drugs. Engineering the pliable surface of the nanoparticles with targeting ligands has led to better homing and internalization of these agents. Targeted delivery of the cyto-toxic drugs to the desired site of action has led to reduction in side effects which are generally caused due to peripheral targeting. Nanoparticles as carriers for diagnosis tools for imaging studies have overcome the shortcomings of the classical methods of imaging. This advancement is essentially of great importance to the field of intra operative imaging. Nanocarriers have made the imaging and drug delivery to even the highly metastasized tumors possible. Recently published data has revealed convincing pre-clinical evidence regarding the potential of active targeted nanoparticles in the diagnosis of CRC and also its therapy. As a result few of these techniques have been translated into early-phase clinical trials. The immense literature and studies in this field clearly suggests that nanocarriers have indeed led to the paradigm shift in the diagnosis and treatment of CRC.

ABBREVIATIONS

CRC-colorectal cancer, 5FU/lV-5-fluorouracil along with leucovorin, FOLFOX-5-fluorouracil along with leucovorin and oxaliplatin, FOLFIRI-irinotecan with 5-fluorouracil and folinic acid, PEG-polyethylene glycol, RES-Reticulo-endothelial system, EPR-enhanced permeability and retention effect, FRs-Folate receptors, FA-Folic acid, HA-Hyluronic acid, CEA-Carcinoembryonic antigen, COX-2-cyclooxygenase-2, NSAIDs-Non-steroidal anti inflammatory drugs, EGFR-Epidermal growth factor receptor, AuNPs-gold nanoparticles, HA-NPs-Hyaluronic acid nanoparticles, SPIONs-superparamagnetic iron oxide nanoparticles, DNA-Deoxyribonucleic acid, P-HA-NPs-polyethylene glycol conjugated hyaluronic acid nanoparticles, MFSN-silica-coated and organic dye-incorporated iron oxide nanoparticles, MRI-magnetic resonance imaging, Ctx-Cetuximab, SSB-superconducting-quantum-interference-device biosusceptometry, PpIX-protoporphyrin IX, 5-ALA-5-aminolevulinic acid, fCNA-folic acid conjugated chitosan nanoparticle, FDA-Food and Drug administration, L-OHP-Oxaliplatin, ILS%-increase in the life span, DAS assay-Dorsal air sac, bPEI-Branched polyethyleneimine, 5-FC-5-Fluorocytosine, DPPC-dipalmitoylphosphatidylcholine, CLX-Celecoxib, CAMNP-acid-coated magnetic nanoparticles, HLs-Hybrid liposomes, DMPC-L α dimyristoylphosphatidylcholine, QT-Quercetin, SLN-Solid lipid nanoparticles, IL-12-Interleukin-12, Th1-T helper cell 1, CS-chitosan, TPP-tripolyphosphate, E-CPNs-Citrus Pectin Nanoparticles, VEGFR-1-vascular endothelial growth factor-1, CENT-Core-excited nanoparticle thermotherapy, MNPs-Magnetic nanoparticles.

CONFLICT OF INTERESTS

Declared None

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