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RECENT TRENDS IN SIRNA DELIVERY FOR TREATMENT OF COLORECTAL CANCER

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ABSTRACT

Colorectal cancer (CRC) is the third most widespread cancer in the world. Currently, chemotherapy is an effective treatment for CRC but acquired multidrug resistance (MDR) due to active drug efflux pumps is the major challenge with chemotherapy. Recently, siRNA (small interfering RNA) therapeutics has getting more attention to overcome the MDR in cancer and other diseases. siRNA is a 21-23 base pair double-stranded RNA having the ability to silence specific genes at the post-transcriptional level. But, clinical practice of siRNA delivery has a limitation due to enzymatic degradation by serum nucleases resulting in poor stability, poor cellular uptake at target site. Nowadays, the development of various nanocarriers for efficient delivery of siRNA is the most challenging and rapidly growing research area. In this review we have summarized, the potential of various nanocarriers such as polymeric nanoparticles, lipid-based nanoparticles, inorganic nanoparticles, layered double hydroxide nanoparticles for siRNA delivery in colorectal cancer treatment.

Keywords: Colorectal cancer, MDR, Nanocarrier, siRNA

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INTRODUCTION

CRC is the third most commonly diagnosed cancer in both men and women. In the United States 95,520 new cases of colon cancer and 39,910 cases of rectal cancer were diagnosed in 2017 [1]. In colorectal cancer, cells from lining of the colon or rectum grow abnormally and out of control over a period of 10 to 20 y. In the initial stage it is non-cancerous and 100% curable. But it slowly develops into adenoma polyp. Further, this one or more adenomas becomes larger and develops into cancer [2]. Current colorectal cancer treatments include surgery, chemotherapy, radiotherapy and combination of them depending on the stage of cancer development [3]. In surgery, tumor with surrounding healthy tissues and lymph nodes are removed. In radiation therapy, high energy beams are used to kill cancer cells which are harmful to healthy cells and causes serious side effects. Most commonly used cytotoxic drugs for the treatment of colorectal cancer are 5-Flurouracil (5-FU) is a thymidylate synthase (TS) inhibitor that inhibits DNA replication, irinotecan inhibits the enzyme topoisomerase, oxaliplatin forms cross-linking DNA which prevents transcription and replication [3, 4]. Cytotoxic drugs are administered through intravenous or oral route in chemotherapy but it shows severe side effects due to its off-target delivery which also harms the normal and healthy cells [2, 5]. Cancer cells gradually acquire MDR to chemotherapeutics due to overexpression of active drug efflux pumps. Other therapeutics used for the treatment of colorectal cancer are monoclonal antibodies, anti-vascular endothelial growth factor-A antibody bevacizumab, anti-epidermal growth factor receptor antibodies cetuximab licensed for use in humans. The combination of monoclonal antibodies with cytotoxic drugs becomes first line treatment for colorectal cancer [5-13]. So, there is a need to develop effective cancer treatments to solve or overcome problems of current cancer treatments and recently it is possible by the use of RNA interference.

RNA interference is a cellular mechanism for gene suppression induced by siRNA. Negatively charged siRNA is 13-16 kDa in molecular weight, double-stranded, 21-23 base pairs in length which acts as a post-transcriptional regulator. Sequence of siRNA is complementary to its target mRNA. According to basic RNA interference mechanism, siRNA binds to the protein complex known as RNA-induced silencing complex (RISC) and guide strand of siRNA binds to the target mRNA and degrade it, resulting reduction in protein level [14-16]. For clinical success, there is a major challenge associated with siRNA delivery at target site and cellular uptake. Some physiological and biological barriers are preventing their delivery at target site. In serum, siRNA is rapidly degraded by serum nucleases resulting in low bioavailability. Chemically unmodified siRNA can trigger an immune response, shows rapid clearance by the reticuloendothelial system (RES) [16-20]. To overcome all these challenges of siRNA delivery there is need of nanocarriers. Here we discussed several nanocarriers like polymeric nanoparticles, liposome and layered double hydroxide nanoparticles (clay materials) used in the treatment of colorectal cancer (fig. 1).

Search criteria

Articles related to colorectal cancer, MDR, nanocarriers for siRNA delivery in CRC were searched on online database i.e. Pubmed, Science Direct for writing this review article. While writing this review, research articles are searched from the year 2013 to 2017.

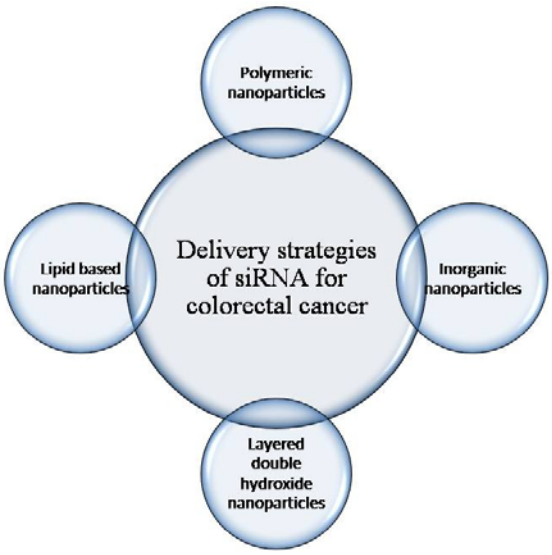

Fig. 1: Delivery strategies of siRNA for colorectal cancer

Gene silencing mechanism of siRNA

RNA interference (RNAi) is a post-transcriptional gene silencing mechanism triggered by siRNA. Generally, two nucleotides

overhangs at both 3' ends of each strands [20]. The siRNA consists of a passenger strand and guide strand. In the cytoplasm, siRNA forms complex with RISC [21, 22]. After binding to RISC, guide strand directed to target mRNA and cleaved by enzyme Argonaut-2 (Argonaute 2-is a member of the Argonaute family of proteins). Thus, there is an interruption of mRNA translation results in blocking the synthesis of the target protein [23-24]. Gene silencing mechanism of siRNA shown in fig. 2.

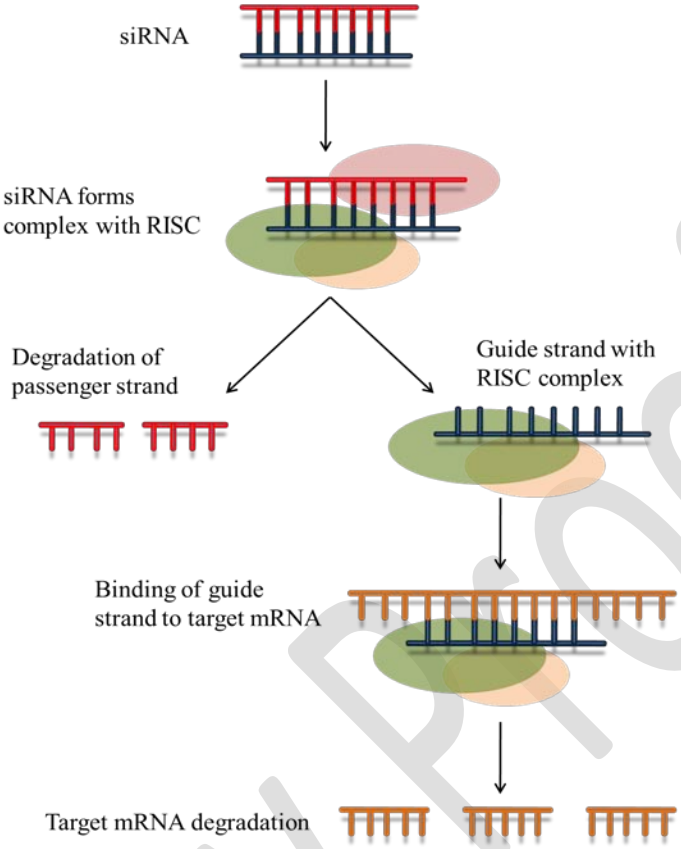

Fig. 1: Gene silencing mechanism of siRNA

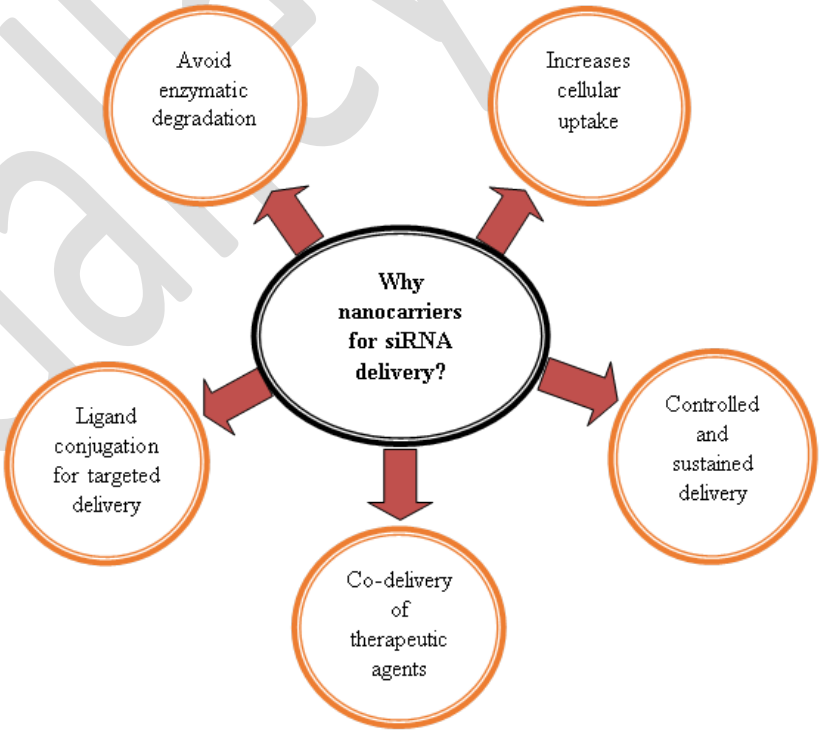

Fig. 3: Advantages of nanocarriers in siRNA delivery

Advantages of nanocarriers for siRNA delivery

Major challenges with siRNA delivery as a therapeutic agent are poor stability, delivery to target site and cellular uptake. In physiological conditions, naked siRNA shows poor stability, short blood circulation time due to enzymatic degradation by serum nucleases and occurs rapid renal clearance because less than 50kDa molecules excreted through the kidney [4, 17]. siRNA is anionic and hydrophilic in nature. So, naked siRNA unable to enter cells by passive diffusion mechanisms. Apart from this, endosomal escape is also a major challenge because siRNA degraded by endosomal enzymes. Bioavailability of siRNA at target site is very low due to off target delivery which consequently affects the site-specific and systemic delivery of siRNA [25-28].

Nanocarrier delivery system protects siRNA from enzymatic degradation during transport to the target site. The conjugation of specific cell targeting molecules such as antibodies, ligands for cell surface receptor, peptides on the surface of nanocarriers and chemical modifications within siRNA enables to recognize a specific type of cell and increases cellular uptake of siRNA at target site. Advantages of nanocarriers in siRNA delivery are shown in fig. 3. Positively charged nanocarrier crossing the negatively charged cell membrane and also able to escape the endosome and release siRNA into the cytoplasm [29, 30].

Table 1: Summary of nanocarriers for siRNA delivery to colorectal cancer

S. No.	Nanocarriers	siRNA	Targeting/Surface modification	Remark	Reference
1.	PDMA-block-poly(ε-caprolactone) micelles	VEGF (vascular endothelial growth factor) gene siRNA	polyethylene glycol (PEG)	Developed micelles passively targeted to tumor regions and synergistically facilitated VEGF silencing and chemotherapy and successfully suppressing tumor growth via a multi-dose therapy. negative magnetic resonance imaging (MRI) contrast agent	34
2.	Poly(lactic-co-glycolic acid) (PLGA)	AHA1 (housekeeping gene)		Developed polymeric nanopharmaceuticals achieve prolonged circulation, tumor accumulation that is uniform throughout the tumor and prolonged tumor-specific knockdown in female homozygous NCR nude mice	35
3.	Cholic acid-polyethylenimine polymer	VEGF	Folic acid	*In vivo* study on Nu/Nu mice showed the lowest cancer cell density and the highest levels of apoptosis and necrosis.	36
4.	Chitosan nanoparticles with different cross linkers such as tripolyphosphate (TPP), dextran sulphate (DS) and poly-D-glutamic acid (PGA)	VEGF gene siRNA		CS-TPP-siRNA nanoparticles showed successful delivery of siRNA within cytoplasm of DLD-1 cells, ionically cross-linked CS-TPP nanoparticles are biocompatible nonviral gene delivery system	37
5.	Carboxymethyl dextran (CMD)–chitosan nanoparticles (ChNPs)	HMGA2 siRNA		CMD-ChNPs reduce expressions of HMGA2 gene, vimentin, MMP-9 and raise E-cadherin expression in HT-29 cell lines.	40
6.	Chitosan and PEGylated chitosan	anti-β-catenin siRNA		Chitosan and PEGylated chitosan nanoparticles containing anti-β-catenin siRNA successfully enter colon cancer cell lines HCT-116 and decreased β-catenin protein levels in cells.	41
7.	Negative lipidoid nanoparticles (NLNs) (mPEG2000-C12/ C14 lipid)	APRIL siRNA		In female BALB/c (nu/nu genotype) athymic mice and female ICR mice NLNs selectively silencing APRIL in the parenchyma of CRC, their uptake proceeded through a lipid raft endocytotic pathway.	46
8.	Liposome-Lipid used Distearoyl-*sn*-glycerophosphocholine (DSPC)	VEGFR2 siRNA		Developed liposomes altering the tumor microenvironment by VEGFR2 blockade has a drastic effect on the intratumoral distribution of nanoparticles in ICR mice (females), Balb/cAJcl nu/nu (nude) mice, C. B-17 SCID) mice (male).	47
9.	Negative lipidoid nanoparticles (mPEG2000-C12/C14 lipid)	a-proliferation-inducing ligand (APRIL) siRNA	mPEG2000-C12/C14 glyceride	*In vivo* experiments on Female ICR mice revealed that a particle size of 90 nm perfectly realized a passive target in a size-dependent manner and did not affect the function of the liver and kidneys by a local delivery method, enema.	48
10.	Nanoliposomes-cationic dioleoyloxypropyl-N,N,N-trimethylammoniumpropane (DOTAP) phospholipid	siRNA against the transcription factor E2F1		SUVs loaded with siE2F1 effective in the down regulation of the target in cultured colon carcinoma cells (HT-29) and in the consequent reduction of cell growth and shows remarkable uptake and target silencing efficiencies in cultured human biopsy of colonic mucosa.	49
11.	layered double hydroxide nanoparticles (LDHs)	Cell Death siRNA		LDHs shows significantly enhanced cytotoxicity to three cancer cell lines MCF-7, U2OS and HCT-116.	50

Characteristics of ideal nanocarriers for sirna delivery in colorectal cancer treatment

As discussed above, to overcome challenges in siRNA delivery there is the requirement of nanocarrier. For effective siRNA delivery, ideal nanocarrier should exhibit the following characteristics-

- All components of nanocarrier should be biocompatible, have low toxicity. Its size must be below 200 nm. It is enough to penetrate cell and avoid kidney filtration which increases cellular uptake [31, 32].
- It must be stable enough in physiological fluids to avoid enzymatic degradation of siRNA. Nanocarrier should have the capability to avoid opsonization and uptake by macrophages. In target cell, endocytosis should occur but siRNA must be able to escape endosome and release into cell cytoplasm [32, 33].

All the above properties are desired and taken into consideration for the development of powerful nanocarrier system for siRNA delivery is shown in (fig. 4).

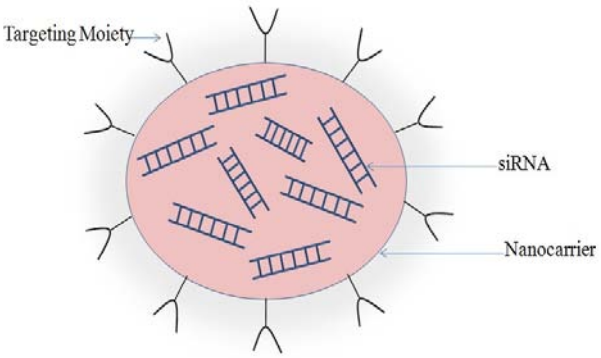

Fig. 4: siRNA loaded targeted nanocarrier

In this review, we summarized siRNA delivery for colorectal cancer treatment by using nanocarriers such as polymeric nanoparticles, lipid nanoparticles, layered double hydroxide nanoparticles (table 1).

Polymeric nanoparticles

Nanocarrier made up of polymers is generally known as polymeric nanocarrier. They are solid, colloidal system and biodegradable in nature which has been widely investigated as drug or gene vectors. Polymeric nanocarriers are having the potential to incorporate high quantity of genomic macromolecules (DNA/RNA etc.) [34]. Incorporation of siRNA into polymeric nanoparticles improves stability of siRNA in serum and prefers controlled release. Their surface can be readily manipulated to improve stability and uptake [35]. Polymers are of two types–natural polymer and synthetic polymer. Chitosan, cyclodextrin, albumin, gelatin are natural polymers for siRNA delivery. Polyethylene glycol (PEG), polyethyleneimine (PEI), Poly (d,l-lactideco-glycolic acid) (PLGA) are synthetic biopolymer used for siRNA delivery [36-39].

Lee *et al.* [34], proposed cationic PDMA-block-poly (ε-caprolactone) (PDMA-b-PCL) micelles as nanocarriers of SN-38 (7-ethyl-10-hydroxycamptothecin), ultra-small superparamagnetic iron oxide nanoparticles (USPIO), siRNA that targets VEGF. The VEGF siRNA conjugated PEG (siRNA-PEG) to improve the siRNA's stability and to prolong its retention time in the blood circulation. Thus, improve the *in vivo* biosafety, by mixed micelles using mPEG-PCL together with PDMA-b-PCL copolymer. The SN-38/USPIO-loaded siRNA-PEG mixed micelleplexes passively targeted to tumor regions and synergistically facilitated VEGF silencing and chemotherapy, thus successfully suppressing tumor growth via a multi-dose therapy. Furthermore, the SN-38/USPIO loaded siRNA-PEG mixed micelleplexes acted as a negative magnetic resonance imaging (MRI) contrast agent in T2-weighted imaging, resulting in a potent tool for the diagnosis and for tracking of the therapeutic outcomes, which disclose its potential as a novel colorectal cancer therapy [34].

Svenson *et al.* [35], developed biocompatible and biodegradable polymeric nanopharmaceuticals (PNPs). PNPs contain PLGA conjugated to PEG for enhanced pharmacokinetics of the nanocarrier, a cation for complexation of siRNA neutral poly(vinyl alcohol) (PVA) to stabilize the PNPs and support the PEG shell to prevent particle aggregation and protein adsorption. The study demonstrated that PNPs accomplish prolonged circulation, tumor accumulation that is consistent throughout the tumor and extended tumor-specific knockdown in female homozygous NCR nude mice [35].

Amjad *et al.* [36], conjugated cholic acid-polyethyleneimine polymer with folic acid, doxorubicin, siRNA forming CA-PEI-FA, D-CA-PEI-FA, D-CA-PEI-FA-S micelles respectively. About 25% doxorubicin released within 24 h at pH 7.4 whereas more than 30% release was observed at pH 5. The presence of FA enhanced micelle anti-tumor activity. The D-CA-PEI-FA and D-CA-PEI-FA-S micelles repressed tumor growth *in vivo* in Nu/Nu mice. Histological analysis discovered that tumor tissues from mice treated with D-CA-PEI-FA or D-CA-PEI-FA-S showed the lowest cancer cell density and the highest levels of apoptosis and necrosis. Similarly, the livers of these mice exhibited the lowest level of dihydropyrimidine dehydrogenase among all treated groups. The lowest serum VEGF (24.4 pg/ml) was observed in mice treated with D-CA-PEI-FA-S micelles using siRNA targeting VEGF. The developed CA-PEI-FA nanoconjugate has the potential to accomplish targeted co-delivery of drugs and siRNA [36].

Raja *et al.* [37] studied stability and efficacy of chitosan nanoparticles with different crosslinkers such as tripolyphosphate (TPP), dextran sulphate (DS) and poly-D-glutamic acid (PGA) used to prepare siRNA loaded CS-TPP/DS/PGA nanoparticles by ionic gelation method. CS-TPP nanoparticles showed better siRNA protection during storage at 4 ℃ and as determined by serum protection assay. The TEM micrographs exposed varied morphology of CS-TPP-siRNA nanoparticles in contrast to irregular morphology displayed by CS-DS-siRNA and CS-PGA-siRNA nanoparticles. All siRNA loaded CS-TPP/DS/PGA nanoparticles showed initial burst release followed by sustained release of siRNA. All the formulations showed low and concentration-dependent cytotoxicity with human colorectal cancer cells (DLD-1) *in vitro*. The cellular uptake studies with CS-TPP-siRNA nanoparticles showed successful delivery of siRNA within cytoplasm of DLD-1 cells. The results revealed that ionically cross-linked CS-TPP nanoparticles are biocompatible nonviral gene delivery system [37].

Siahmansouria *et al.* [40] used carboxymethyl dextran (CMD)–chitosan nanoparticles (ChNPs) for encapsulation of HMGA2 siRNA and DOX. Then the efficiency of the simultaneous delivery on viability and gene expression evaluated on HT-29 cell lines. Cell viability and relative mRNA expression were evaluated by MTT assay and real-time PCR respectively. The prepared ChNPs had high encapsulation efficiency of siRNA and drug. These developed formulations are stable against serum and heparin. ChNP/siRNA/DOX/CMD was more efficient to induce tumor cell death and also might considerably reduce the expressions of HMGA2, vimentin, MMP-9 and raise E-cadherin expression. Their results discovered that dual delivery of a key gene siRNA and anticancer drug have large effect on the treatment of colorectal cancer [40].

Rudzinski *et al.* [41] developed chitosan and PEGylated ChNPs, 100-150 nm in diameter, encapsulating anti-β-catenin siRNA for transfection into colon cancer cells (HCT-116). Up to 97% siRNA were encapsulated and entry of fluorescently-tagged siRNA observed under confocal microscopy. Western blot analysis showed that both chitosan and PEGylated ChNPs containing anti-β-catenin siRNA decreased β-catenin protein levels in cultured HCT-116 colon cancer cells. These results indicated that nanoparticles made with Ch and PEGylated Ch can successfully enter colon cancer cells and decrease the level of a protein that promotes tumor progression [41].

Lipid-based nanoparticles

Lipid-based carriers have been used successfully to transport siRNA to proposed sites in the endothelium, RES, and solid tumors. Normally, variety of lipid-based siRNA delivery systems are available like liposomes, nanoemulsions, and solid lipid

nanoparticles but the most recurrently used systems are (a) Liposomal system in which encapsulation of siRNA within the vesicles is made up of a phospholipid bilayer. (b) Lipoplexes, in which cationic lipids complexes with siRNA like {1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE), N-(N-[1-(2,3-dioleyloxy) propyl]-N), N, N trimethyl ammonium chloride (DOTMA), N, N-dioleyl-N, N-dimethylammonium chloride (DODAC) and obtain formation of complexes at nanoscale [39, 42-45]. Liposomes are globular vesicles made up of an aqueous core and bilayers of phospholipid with lipids, sterols and are having biocompatible, biodegradable characteristics. The amine groups of phospholipids share electrostatic interaction with negatively charged siRNA and helps to deliver at the target region [39].

Ding *et al.* [46], developed negative lipidoid nanoparticles (NLNs) encapsulating siRNA for selectively silencing APRIL in the parenchyma of CRC. Uptake of developed formulation proceeded through a lipid raft endocytotic pathway. Their *in vivo* study on female BALB/c (nu/nu genotype) athymic mice and female ICR mice reveals that APRIL to be a latent anti-CRC target and suggests that the application in other therapeutics may be possible [46].

Yamamoto *et al.* [47], reported the enhancement in the intratumoral distribution of liposome by VEGFR2 inhibition reliant on the vascular type of the tumor. VEGFR2 inhibition found to change the tumor microenvironment, including heparan sulfate proteoglycans (HSPGs). The effect of the size of nanoparticles indicated that VEGFR2 inhibition improved the penetration of nanoparticles through the vessel wall. Study suggests that a combination of anti-angiogenic therapy and delivery via the EPR effect useful and that changing the tumor microenvironment by VEGFR2 obstruct has a strong effect on the intratumoral distribution of nanoparticles in ICR mice [(females), Balb/cAJcl, nu/nu (nude) mice, C. B-17 SCID) mice (male)] [47].

Ding *et al.* [48], described NLNs delivery system, providing entrapment-based transfection agents for local delivery of siRNA to the colorectal cancer. Nanoparticles synthesized with lipidoid material 98N12-5(1), mPEG 2000-C12/C14 glyceride and cholesterol at the desired molar ratio to understand the anionic surface charge of particles. *In vivo* experiments on female ICR mice revealed that a particle size of 90 nm perfectly realized a passive target and did not affect the function of the liver and kidneys by enema. The uptake of NLNs internalized through a lipid raft endocytotic pathway with low cytotoxicity, strong biocompatibility and high efficacy [48].

Bochicchio *et al.* [49], developed nanoliposomes loaded with a siRNA against the transcription factor E2F1 (siE2F1), delivered to human colorectal adenocarcinoma cell lines (HT-29) and to intestinal human biopsies. siE2F1 loaded nanoliposomes created by ultrasound assisted technique with 40 nm particle size (Small Unilamellar Vesicles, SUVs) and 100% siRNA encapsulation efficiency. By using suitable ultrasonic duty cycle treatments easily produce particles in the nanometric scale than other production methods siE2F1-loaded SUVs demonstrated very low cytotoxicity in cells when compared to a commercial transfection agent. SUVs loaded with siE2F1 effective in the down-regulation of the target in cultured colon carcinoma cells and in the subsequent reduction of cell growth and shows remarkable uptake and target silencing efficiencies in cultured human biopsy of colonic mucosa. siE2F1-SUVs have the possible to contribute to the development of novel efficient inflammatory bowel disease-associated colorectal cancer therapies for a future modified medicine [49].

Clay material

Li *et al.* [50], developed layered double hydroxide nanoparticles (LDHs) simultaneously delivered 5-FU and All stars Cell Death siRNA for cancer treatment. By taking benefit of the LDH anion exchange capacity to intercalate 5-FU into its interlayer spacing and load siRNA on the surface of LDH nanoparticles. The combination of CD-siRNA and 5-FU with LDH particles shows considerably improved cytotoxicity to three cancer cell lines MCF-7, U2OS and HCT-116 as compared to the single treatment with either CD-siRNA or 5-FU. This improvement is possibly a result in mitochondrial damage process. Study revealed that co-delivery of siRNA and an anticancer drug by LDHs has great potential to overcome the drug resistance and improve cancer treatment [50].

CONCLUSION

Researchers reported various nanocarriers for siRNA delivery such as polymeric nanoparticles, lipid-based nanoparticles are capable of shielding the siRNA from enzymatic degradation, augment cellular uptake by surface modification, escape from renal clearance and demonstrate the sustained release of siRNA at the target site. *In vitro* and *in vivo* results reported by researchers depicted that siRNA loaded nanocarriers for the treatment of CRC are capable of enhancing antitumor activity by silencing the specific gene.

Future perspectives

Future directions for the development in this field must include the discovery of new target RNA genes to increase apoptosis in cancer cells and killing the cancer cells by reducing side effects in normal and healthy cells, precise accumulation of nanoparticles exclusively in tumor cells, the real-time imaging and monitoring of treatment effects *in vivo*. Multidisciplinary research studies will direct the development of highly efficient and safer RNAi-based therapeutics in clinical trials. In preclinical trials, active targeting may be more prevalent in the future design of nanocarriers for siRNA delivery and co-delivery.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICTS OF INTERESTS

Declared none

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