International Journal of Nanomedicine

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REVIEW

Antibody-Functionalized Nanoformulations for Targeted Therapy of Colorectal Cancer: A Systematic Review

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Abstract: The failure of chemotherapeutic treatment in colorectal cancer (CRC), the second most mortal cancer worldwide, is associated with several drug limitations, such as non-selective distribution, short half-life, and development of multiple resistances. One of the most promising strategies in CRC therapy is the development of delivery systems based on nanomaterials that can transport antitumor agents to the tumor site more efficiently, increasing accumulation within the tumor and thus the antitumor effect. In addition to taking advantage of the increased permeability and retention effect (EPR) of solid tumors, these nanoformulations can be conjugated with monoclonal antibodies that recognize molecular markers that are specifically over-expressed on CRC cells. Active targeting of nanoformulations reduces the adverse effects associated with the cytotoxic activity of drugs in healthy tissues, which will be of interest for improving the quality of life of cancer patients in the future. This review focuses on in vitro and in vivo studies of drug delivery nanoformulations functionalized with monoclonal antibodies for targeted therapy of CRC.

Keywords: nanoformulation, colon carcinoma, monoclonal antibody, 5-fluorouracil, targeted therapy

Introduction

Colorectal cancer (CRC) accounts for the third highest incidence of cancer and the second highest mortality in the world.[1,2] Changes in lifestyle and dietary patterns, including increased consumption of processed food, sedentarism, smoking, alcohol, and low intake of fruits, vegetables, and calcium, among others, have been related to a significant increase in the incidence of CRC in recent years.[3] Moreover, far from improving, CRC mortality is estimated to increase by more than 60.0% by 2035.[4]

The treatment of choice for non-metastatic CRC is surgery. However, the management of metastatic CRC, which occurs in 50% of patients,[5] consists of surgical resection of the tumor (and metastases when possible), together with chemotherapy, radiotherapy and targeted therapy. 5-fluorouracil (5-FU), oxaliplatin (OXA) and irinotecan (IRI) are the chemotherapeutics of first choice, and can be used alone or in combination regimens such as FOLFOX (5-FU/leucovorin/OXA), FOLFIRI (5-FU/leucovorin/IRI) and FOLFOXIRI (5-FU/leucovorin/OXA/IRI).[6] Unfortunately, these drugs have numerous side effects on proliferating cells, such as those found in the digestive tract, hair follicles or hematopoietic progenitors. In fact, FOLFOXIRI has been significantly associated with the development of grade 3 and 4 neurotoxicity and neutropenia, limiting its therapeutic success due to treatment discontinuation by patients.[7] Likewise, the search for CRC cell-specific biomarkers has allowed the development of targeted therapy; including agents acting against EGFR (eg, cetuximab and panitumumab), as well as against VEGF (eg, bevacizumab and aflibercept).[8,9] These biomarkers, in

Received: 31 March 2022
Accepted: 21 August 2022
Published: 31 October 2022

turn, can be used for the generation of new strategies for targeted drug delivery to tumor cells. However, all these therapeutic advances have failed to increase the survival of patients with advanced disease which remains below 15%.[10]

Treatment failure of metastatic CRC has various causes, including adverse effects of chemotherapy, drug non-specificity, and drug resistance mediated by ABC (ATP-binding cassette) transporters, among others.[11] Thus, the development of new strategies to improve the treatment of these patients is a priority. In this context, nanomedicine represents a promising field for the development of new antitumor nanodrugs that could be released locally at the tumor site, overcoming the limitations of conventional chemotherapy and improving adherence to treatment and the quality of life of patients.[12]

The most widely used nanoformulations in cancer therapy include polymeric nanoparticles (NPs), lipid nanoformulations (liposomes and micelles) and inorganic NPs. These nanoformulations improve stability, solubility, and drug half-life, and are able to increase accumulation within the tumor due to the EPR effect of solid tumors, which is closely related to passive targeting and relies on paracellular transport of the nanoformulations through compromised blood vessels and subsequent non-specific interaction with tumor cells. However, their effectiveness is compromised due to high inter- and intra-tumor variability.[12–14] Furthermore, some of these nanodrugs block resistance mechanisms that prevent the elimination and/or degradation of the drug by the tumor cell.[15] Specifically, in CRC therapy, a wide variety of nanoformulations are being used, including liposomes and polymeric NPs,[16] which have shown high efficacy. This is the case with liposomes associated with doxorubicin (DOX) and curcumin (co-encapsulation), which increased antitumor efficacy in CRC in vivo models,[17] and with polymeric NPs loaded with Nutlin-3a and granulocyte colony stimulating factor- macrophages (GM-CSF), which have recently shown enhanced antiproliferative effects against CRC.[18] Likewise, some nanoformulations against CRC attempt to avoid multidrug resistance (MDR) mechanisms. For instance, Jiang et al used nanocomposites based on a gold nanorod core-shell associated with DOX and functionalized with poly-histidine and d-α-tocopherol polyethylene glycol 1000 succinate (TPGS) that inhibited p-glycoprotein.[19] Clinical trials are the final step in the use of these nanoformulations in CRC, as is the case with TKM-080301, a lipid NP loaded with an siRNA against the PLK1 protein, or CRLX101, a PEGylated cyclodextrin NP with camptothecin.[16,20,21] These trials may prove the usefulness of these systems to improve the prognosis of CRC patients.

Specific interactions with tumor cells can be achieved by active targeting nanoformulations, designed with specific ligands that recognize with high affinity tumor cell receptors. This active targeting allows i) to improve the retention of passively accumulated nanoformulations due to ligand-receptor interaction, and ii) to provide specific interaction with tumor cells by reducing interactions with non-targeted cells.[14] In this context, the functionalization of NPs by using monoclonal antibodies —the most widely used targeting-ligands[22] that allow their targeting to the tumor cell represents a great opportunity for the improvement of oncological treatment. This tissue- or cell-specific delivery occurs through the development of antibody-NP conjugates (ANCs) that bind specifically to the cell type of interest significantly reducing their non-specificity and toxicity in non-tumor tissues.[23] The use of ANCs in CRC has emerged as a field of interest. The EGFR tyrosine kinase receptor, involved in tumor growth and progression,[24] is one of the surface molecules most commonly used to target ANCs in CRC[25,26] and other types of cancer (eg, prostate, skin cancer and lung cancer).[27–29] In fact, a patent has already been published for cetuximab (anti-EGFR) bound to carbon quantum dots, which showed a high targeting capacity in EGFR-overexpressing tumor cells.[30,31] New therapies targeting cancer stem cells (CSC), which appear to be responsible for resistance to chemotherapy, radiotherapy and the development of metastases, are under investigation.[32] In fact, novel nanovehicles that specifically target this cell population have been developed. For example, a recent study used functionalized DCLK1 folic acid conjugated hesperetin encapsulated in chitosan NPs to selectively target colorectal CSC.[33] The stem cell biomarker CD133 has become a way of targeting colon,[34] breast[35] and ovarian stem cells,[36] among others. Recently, this ligand has been used by Mohd-Zahid et al to synthesize PEGylated gold NPs that significantly increased intracellular drug (5-FU) accumulation in HCT116 CRC cells.[37]

The main objective of this systematic review is to analyze all the recent published studies on NPs, liposomes and micelles functionalized with monoclonal antibodies and associated to a molecule with antitumor activity against CRC. This review summarizes the main antibody-NP conjugates used in human CRC, including in vitro and in vivo assays, and supports the need for further studies to understand their main mechanism of action and their application in patients with CRC.

Materials and Methods

Study Eligibility

The purpose of this systematic review is to evaluate the most recent scientific publications containing information on the therapeutic efficacy of antitumor agents carried by NPs, liposomes or micelles functionalized with monoclonal antibodies or fragments of them in CRC. The systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria.[38] Literature published more than 10 years ago was considered obsolete. In addition, according to the Burton-Kebler index for obsolescence, more than half of the scientific publications on this topic were included.[39]

Inclusion and Exclusion Criteria

This review included studies written in English and with accessible full text, published between May 2011 and May 2021, about NPs, liposomes or micelles that i) target cells of interest with monoclonal antibodies or fragments of them; and ii) function as antitumor molecule delivery agents against CRC. Studies with insufficient information on the data provided, as well as systematic reviews, reviews, meta-analyses and editorials were excluded.

Data Sources

The literature search was performed in the following electronic databases: Pubmed, SCOPUS and Web of Science. First, the medical subject heading (MeSH) terms were established; "Colorectal Neoplasms", "Nanoparticles", "Liposomes", "Micelles" and "Antibodies" were used as descriptive terms. The final search strategy was (("Colorectal Neoplasms" [MeSH Terms] OR (("colon" [Title/Abstract] OR "colorectal" [Title/Abstract] OR "colonic" [Title/Abstract]) AND ("cancer*" [Title/Abstract] OR "tumor*" [Title/Abstract] OR "tumour*" [Title/Abstract] OR "neoplasm*" [Title/Abstract] OR *carcinoma* [Title/Abstract]))) AND (("nanoparticles" [MeSH Terms] OR "nanoparticle*" [Title/Abstract] OR "nanoconjugate*" [Title/Abstract] OR "liposomes" [MeSH Terms] OR "liposome*" [Title/Abstract] OR "micelles" [MeSH Terms] OR "micelle*" [Title/Abstract]) AND ("antibodies" [MeSH Terms] OR "antibod*" [Title/Abstract]))). Slight modifications were made to adapt the search strategy to the different databases.

Study Selection

Two of the authors (C.L. and A.C.) conducted the literature search. In the first stage of the review, the titles and abstracts of the studies were screened and those meeting the inclusion criteria were selected for further review. In the second stage, the authors reviewed the full text of all the selected articles according to the previously established inclusion and exclusion criteria.

Data Extraction

After the selection process of publications was completed, the same two authors independently reviewed and extracted the data from the selected studies. According to Cohen's Kappa statistical test,[40] which exceeded 0.8, there was concordance between the two investigators.[41] Discrepancies were resolved by consensus between C.L. and A.C., or between two other authors if necessary. The quality of the selected publications was determined by means of a specific questionnaire. The extracted data are summarized in Tables 1–4 and have been classified according to the type of nanoformulation used. In order to facilitate the understanding of the selected studies, details on the following variables have been included: antitumor agent transported, monoclonal antibody used for functionalization, type of nanoformulation, most relevant in vitro and in vivo results, and publication reference.

Results and Discussion

Study Description

After the initial literature search in the different electronic databases, 778 articles were retrieved following the inclusion and exclusion criteria. Titles and abstracts were reviewed to exclude articles that did not meet the selection criteria as well as duplicates. After full-text review of the 80 articles selected, 36 did not fit the subject of the review, 4 were not

Table 1 Antiproliferative Activity of Lipid Nanoformulations in Colorectal Cancer Models

Antitumor Agent	Antibody	Nanocarrier	Types of Study in CRC Models		Main Results		Reference
			In vitro	In vivo	Compared to Non-Targeted	Compared to Free Antitumor Agent	
DOX (Doxil)	Anti-CD133	Doxil ®	Cytotoxicity assay (HT-29)	-	3-fold higher cytotoxicity	Not evaluated	[34]
5-FU	Anti-FZD10	PEGylated liposomes	Cytotoxicity and migration assays (Caco-2 and CoLo-205)	-	Not evaluated	2.6-fold higher cytotoxicity at lower concentrations	[43]
Leucine-Leucin-Norleucinal (LLNle)	Cetuximab	PEGylated liposomes	Cytotoxicity assay (HCT-116)	-	Lower cytotoxicity	No differences in cytotoxicity	[44]
RA-V and RX-0047 (RA/RV)	Anti-DR5	PEGylated liposomes	Cytotoxicity, apoptosis (HCT-116 and HT2-9) and overcoming tumor hypoxia assays (HCT-116)	HCT-116 tumor-bearing mice	Not evaluated	In vitro: higher cytotoxicity compared to both compounds individually; In vivo: higher cytotoxicity compared to both compounds individually	[45]
PTX	Anti-PD-L1	Cerasomes	Cytotoxicity (CT- 26)	CT-26 tumor-bearing mice	In vitro: no differences in cytotoxicity; In vivo: higher tumor growth inhibition	In vitro: no differences in cytotoxicity; In vivo: higher tumor growth inhibition	[46]
5-FU	Anti-EGFR	PEGylated liposomes	Cytotoxicity assay (HCT-116)	HCT-116 tumor-bearing mice	Not evaluated	In vitro: 2 to 3-fold higher cytotoxicity; In vivo: 1,4-fold higher tumor growth inhibition	[47]
MG85	Cetuximab	PEGylated liposomes	Cytotoxicity assay (HCT-116)	-	4-fold higher cytotoxicity	2-fold higher cytotoxicity	[48]
OXA	Anti-TRAIL	Solid lipid NPs	Cytotoxicity assay (HT-29)	-	Higher cytotoxicity	8-fold higher cytotoxicity	[49]
DOX (Doxil)	Anti-CD44	Doxil ®	Cytotoxicity assay (C-26)	C-26 tumor-bearing mice	In vitro: 2-fold higher cytotoxicity; In vivo: higher tumor inhibition efficacy and survival rates	Not evaluated	[42]

OXA	Cetuximab and Fab' fragment of Cetuximab	PEGylated liposomes	Cytotoxicity assay (HCT-116, HT-29, SW-480 and SW-620)	SW480 tumor-bearing mice	In vitro: higher cytotoxicity; In vivo: higher tumor growth inhibition, higher delay in tumor growth in Fab'-functionalized nanocarrier	In vitro: higher cytotoxicity; In vivo: higher tumor growth inhibition	[50]
Celecoxib	Cetuximab	PEGylated liposomes	Cytotoxicity assay (HT-29 and SW-620)	-	Higher cytotoxicity	No differences in cytotoxicity	[51]
5-FU	Anti-ITGB6	PEGylated liposomes	Cytotoxicity and apoptosis assays (SW-480β6 and HT-29)	HT29 and SW480 tumor-bearing mice	In vitro: higher cytotoxicity, 1.5-fold higher cellular apoptosis; In vivo: 3-fold higher tumor growth inhibition, 1.5 to 1.7-fold higher cellular apoptosis	In vitro: higher cytotoxicity, 2.4-fold higher cell apoptosis; In vivo: higher tumor growth inhibition	[52]
DOX	Anti-MUC-1	PEGylated liposomes-ICG	-	HT29 tumor-bearing mice	Not evaluated	Not evaluated	[53]
DOX	Anti-VEGFR	PEGylated liposomes	-	HT29 tumor-bearing mice	No differences in tumor inhibition efficacy	Not evaluated	[54]

Abbreviations: FZD10, Frizzled Class Receptor 10; PEG, polyethilene glycol; DR5, Death Receptor 5; PD-L1, Programmed Death-ligand 1; EGFR, Epidermal Growth Factor Receptor; TRAIL, TNF-Related Apoptosis-inducing Ligand; NPs, nanoparticles; ITGB6, Integrin Subunit Beta-6; MUC-1, Mucin 1; ICG, indocianine green; VEGFR, Vascular Endotelial Growth Factor Receptor.

https://doi.org/10.2147/IJN.S368814

Table 2 Antiproliferative Activity of Polymeric Nanoformulations in Colorectal Cancer Models

Antitumor Agent	Antibody	Nanocarrier	Types of Study in CRC Models		Main Results		Reference
			In vitro	In vivo	Compared to Non-Targeted	Compared to Free Antitumor Agent	
Triptolide	Anti-HER-2	PGA L-Phe NPs	Cytotoxicity, cell cycle and apoptosis assays (HT-29)	HT-29 tumor-bearing mice	Not evaluated	In vitro: 3-fold higher cytotoxicity, higher cell cycle arrest in G1-S phase and 2-fold higher apoptosis; In vivo: higher tumor inhibition efficacy and survival rates	[60]
5-FU	Cetuximab	PEGylated PLA NPs	Cytotoxicity and apoptosis assays (SW620)	SW620 tumor-bearing mice	Not evaluated	In vitro: higher cytotoxicity	[61]
Curcumin	Cetuximab	Citrus pectin-chitosan NPs	Cytotoxicity, apoptosis and cell cycle assays (Caco-2 and HCT-116)	-	1,4-fold higher cytotoxicity, 1.8-fold higher cycle arrest in G2/M phase	29.8 and 30-fold higher cytotoxicity in Caco-2 and HCT-116, respectively	[63]
PTX	Anti-CEA	PEGylated PLGA NPs	Cytotoxicity assay (Caco-2 andSW480)	-	No difference in cytotoxicity	Lower cytotoxicity	[59]
IRI	Anti-CD133	mPEG–PCL/ mal-PEG –PCLNPs	Cytotoxicity (HT-29 and HCT-116) and colony formation assays (HCT-116)	HCT-116 tumor-bearing mice	In vitro: higher cytotoxicity in HCT116, higher inhibition of colony formation; In vivo: higher tumor growth inhibition, higher inhibition of tumor relapse	In vitro: no differences in cytotoxicity; In vivo: higher tumor growth inhibition, higher inhibition of tumor relapse	[62]
Camptothecin	Anti-DR5	PEGylated PLGA NPs	Cytotoxicity (RKO, LoVo and HT-29) and cell death assays (HCT-116)	HCT-116 tumor-bearing mice	In vitro: higher cellular apoptosis, higher cytotoxicity; In vivo: higher tumor inhibition efficacy	Not evaluated	[65]
Camptothecin	Conatumumab	PLGA NPs	Cytotoxicity and cell death assays (HCT-116)	-	5-fold higher cytotoxicity, higher cellular apoptosis	4-fold higher cytotoxicity, higher cellular apoptosis	[64]

Abbreviations: HER-2, Human Epidermal Growth Factor Receptor 2; PGA, poly(glycolic acid); NPs, nanoparticles; PEG, polyethylene glycol; PLA, poly(lactic acid); CEA, Carcinoembryonic Antigen; PLGA, poly(lactic-co-glycolic acid); PCL, polycaprolactone; DR5, Death Receptor-5.

Table 3 Antiproliferative Activity of Inorganic Nanoformulations in Colorectal Cancer Models

Antitumor Agent	Antibody	Nanocarrier	Types of Study in CRC Models		Main Results		Reference
			In vitro	In vivo	Compared to Non-Targeted	Compared to Free Antitumor Agent	
5-FU	Anti-CD133	PEGylated gold NPs	Cytotoxicity assay (HCT-116)	-	Higher cytotoxicity	Not evaluated	[37]
5-FU	Anti-EGFR	Gold NPs	Cytotoxicity and apoptosis assays (HCT-116 and HT-29)	-	No differences in cytotoxicity, higher apoptosis	No differences in cytotoxicty, higher apoptosis	[26]
DOX	Anti-EGFR	Graphene Quantum Dots with PEI	Cytotoxicity assay (HCT-116)	HCT-116 tumor-bearing mice	In vitro: higher cytotoxicity	In vitro: lower cytotoxicity; In vivo: no difference in tumor inhibition efficacy, higher safety	[71]
[Zn(DION)2]Cl2—ZnD	Cetuximab	Multifunctional gold NPs	Cytotoxicity assay (HCT116)	HCT-116 DR tumor-bearing mice	In vitro: no differences in cytotoxicity; In vivo: no differences in tumor growth inhibition	In vitro: higher cytotoxicity; In vivo: no differences in tumor growth inhibition	[72]
RBT	Bispecific antibodies (anti-CD16 and anti-CEA)	PEGylated hollow mesoporous ruthenium NPs	Cytotoxicity and apoptosis (CT26-CEA and HIEC-6), ROS (CT26-CEA) and antitumor efficacy in spheroid assays (CT26-CEA)	CT26-CEA tumor-bearing mice	In vivo: higher tumor growth inhibition	In vitro: higher cytotoxicity, higher cellular apoptosis; In vivo: higher tumor growth inhibition	[69]
DOX	Anti-PD-L1	Gold NPs	Cytotoxicity, apoptosis, ROS and cell cycle assays (CT-26)	-	1,6-fold higher cytotoxicity, 1,8-fold higher cellular necrosis, higher ROS generation	2-fold higher cytotoxicity, 1.5-fold higher cellular necrosis, 2.6-fold higher ROS generation	[73]
PTX	Cetuximab	Nanodiamond	Cytotoxicity and apoptosis assays (RKO, HCT116 and SW620)	RKO tumor-bearing mice	In vivo: higher tumor growth inhibition and cellular apoptosis	Not evaluated	[74]
TS265	Anti-EGFR	Multifunctional gold NPs	Cytotoxicity assay(HCT116)	HCT-116 tumor-bearing mice	In vitro: no differences in cytotoxicity; In vivo: higher tumor growth inhibition	In vitro: 1.5-fold higher cytotoxicity	[75]
OXA	Anti-DR5	Gold NPs	Cytotoxicity and apoptosis assays (HCT1166)	HCT-116 tumor-bearing mice	In vitro: higher cellular apoptosis; In vivo: higher tumor growth inhibition	In vitro: 3.2-fold higher cytotoxicity, 3-fold higher cellular apoptosis; In vivo: higher tumor growth inhibition, higher safety	[76]
Mifepristone	Anti-EpCAM	Mesoporous silica NPs	Cytotoxicity and cell cycle assays (HT29 and SW620)	SW620 tumor-bearing mice	In vitro: higher cytotoxicity, 1,3-fold higher inhibition of adhesion to endothelial cells, higher cell cycle arrest in G0/G1 phase; In vivo: higher inhibition of lung metastasis	In vitro: 1.3-fold higher inhibition of adhesion to endothelial cells; In vivo: higher inhibition of lung metastasis	[68]

Abbreviations: PEG, polyethylene glycol; NPs, nanoparticles; EGFR, Epidermal Growth Factor Receptor; PEI, polyethylenimine; CEA, Carcinoembryonic Antigen; PD-L1, Programmed Death-ligand 1; DR5, Death Receptor-5; EpCAM, Epithelial Cell Adhesion Molecule.

https://doi.org/10.2147/IJN.S368814

https://doi.org/10.2147/IJN.S368814 DovePress

Table 4 Antiproliferative Activity of Hybrid and Peptide Nanoformulations in Colorectal Cancer Models

Antitumor Agent	Antibody	Nanocarrier	Types of Study in CRC Models		Main Results		Reference
			In vitro	In vivo	Compared to Non-Targeted	Compared to Free Antitumor Agent	
DOX	Cetuximab	Bovine serum albumin NPs	Cytotoxicity assay (RKO and LS174T)	RKO tumor-bearing mice	In vitro: higher cytotoxicity; In vivo: increase in tumor inhibition efficacy	In vitro: no differences in cytotoxicity; In vivo: no differences in tumor growth inhibition, higher survival rates	[83]
5-FU	Cetuximab	Mesoporous silica NPs coated with PEGylated liposome	Cytotoxicity, cell cycle and apoptosis assays (HCT-116 y SW620)	HCT-116 and SW620 tumor-bearing mice	In vitro: higher cytotoxicity, higher cycle arrest in S phase, 3,3-fold higher cellular apoptosis	In vitro: 5.8-fold higher cytotoxicity, 2.3-fold higher cycle arrest in S phase, 1,9-fold higher cellular apoptosis	[81]
Carfilzomib	Anti-EpCAM	PEGylated Ternary polypeptide NPs	Cytotoxicity assay (DLD-1)	-	No difference in cytotoxicity	4.5-fold higher cytotoxicity	[66]
Niclosamide	Fab-CD44v6 (antibody fragment)	Polymeric micelles	Cytotoxicity and colonospheres formation assays (HCT-116)	HT29 tumor-bearing mice	In vitro: 2,8-fold higher cytotoxicity, higher inhibition of colonosphere formation; In vivo: no differences in tumor growth inhibition, higher cytotoxicity in CTC	In vitro: 3.5-fold higher cytotoxicity, higher inhibition of colonosphere formation; In vivo: no differences in tumor growth inhibition, higher cytotoxicity in CTC	[80]
5-FU	Anti-SMC2	Polymeric micelles	Cytotoxicity and colonosphere assays (HCT-116)	-	Higher cytotoxicity in adherent cells	No differences in cytotoxicity in adherent cells, 4-fold higher cytotoxicity in colonospheres	[86]
Gambogic acid	Bispecific recombinant protein anti-EGFR-iRGD	Red blood cell membrane-coated PLGA NPs	Cytotoxicity and apoptosis assays (HT29)	Caco-2 tumor-bearing mice	Not evaluated	In vitro: 1.3-fold higher cytotoxicity, no differences in cellular apoptosis; In vivo: no differences in tumor growth inhibition, higher survival rates	[85]
PTX	Cetuximab	PEGylated PLGA-polymeric micelles	Cytotoxicity assay (HCT-116 and HCT-8)	-	4,4-fold higher cytotoxicity	16.5-fold higher cytotoxicity	[84]
DOX	Anti-DR4	PEGylated PLGA-gold NPs	Cell viability (DLD-1)	DLD-1 tumor-bearing mice	Not evaluated	In vivo: higher tumor growth inhibition, higher safety	[82]

Abbreviations: NPs, nanoparticles; PEG, polyethylene glycol; EpCAM, Epithelial Cell Adhesion Molecule; SMC2, Structural Maintenance of Chromosomes 2; EGFR, Epidermal Growth Factor Receptor; RGD, arginine–glycine–aspartic acid peptide; PLGA, poly(lactic-co-glycolic acid); DR4, Death Receptor-4.

available in full text, and 1 was excluded due to low quality. Finally, 39 articles were included in this systematic review. The flow diagram of the search process is presented in Figure 1.

Most of the studies focused on the evaluation of the cytotoxic effects of antitumor agents delivered by lipid nanoformulations (14 articles) and inorganic nanoformulations (10 articles). The remaining studies used polymeric, peptide or hybrid nanoformulations (ie, derived from combinations of the previous ones). In addition, 28 articles analyzed carrier nanoformulations of conventional antitumor agents such as DOX (10 articles), 5-FU (8 articles) and paclitaxel (PTX) (4 articles). Moreover, most of the functionalizations used antibodies against the EGFR (15 articles), with cetuximab being the most widely used. The predominant targeting of EGFR, a transmembrane receptor of the tyrosine kinase family that mediates cell signalling cascades involved in cell proliferation, angiogenesis and apoptosis, is explained by its overactivation in numerous types of cancer, including CRC. Therefore, the use of monoclonal antibodies that act as selective competitors by blocking the binding of endogenous ligands and inhibiting the signalling cascade is a strategy under extensive study.[24]

Lipid Nanoformulations

Of the 39 articles included in this review, 14 analyzed the antitumor effect of lipid nanocarriers in CRC (Table 1), including liposomes coated with PEG chains (11 articles), the chemotherapeutic drug Doxil® (2 articles), and a cerasome (1 article). Of note, studies with Doxil® targeted against CD133 +[34] and CD44 + tumor cells[42] -both of which are clusters of differentiation associated with

CSC of CRC-[32] showed a 3-fold and 2-fold improvement in cell cytotoxicity compared to non-targeted Doxil®, respectively.

Regarding cytotoxic drugs transported by lipid nanocarriers, DOX (5 articles) and 5-FU (3 articles) were the most commonly used. PEGylated liposomes loaded with 5-FU and functionalized with anti-FZD10, anti-EGFR and anti-ITGB6 were tested in CRC. Specifically, of the 14 articles on lipid nanoformulations, five were directed against EGFR. Interestingly, the use of anti-EGFR and anti-ITGB6 –which recognizes integrin β6, involved in invasion and metastasis of CRC- nanoformulations showed significant enhancement of in vitro cytotoxicity, including reduced tumor growth in murine CRC models compared to free 5-FU.[47,52] Furthermore, anti-FZD10 nanoformulations targeting the FZD10 receptor of the WNT signaling cascade showed enhanced antitumor effect of the drug at low concentrations (1–2 μM).[43] PTX,[46] OXA,[50] Celecoxib,[55] Z-Leucinyl-Leucinyl-Norleucinal tripeptide,[44] RA-V cyclopeptide[45] and MG85 complex[48] were also analyzed.

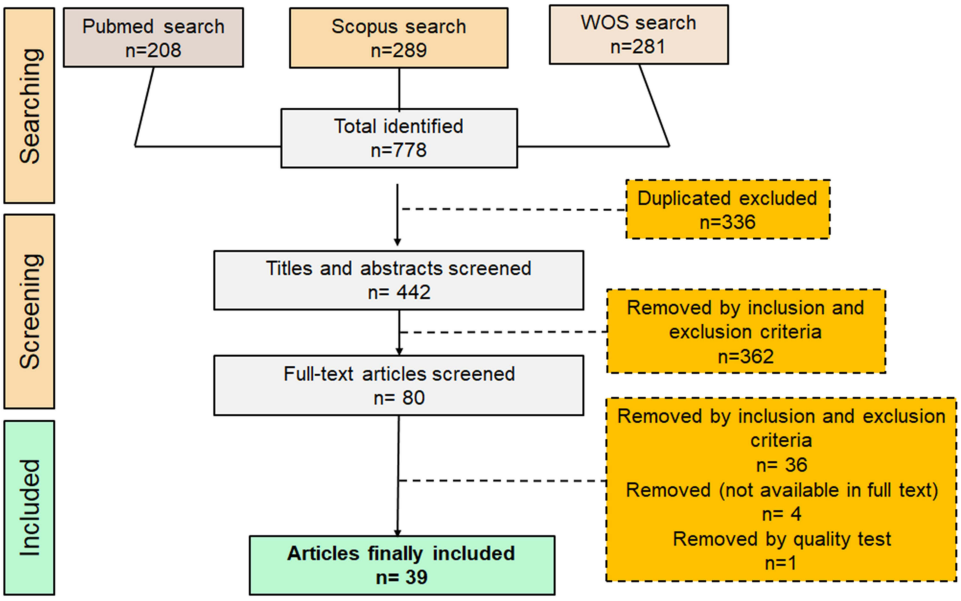

Figure 1 Flow diagram of articles included in this systematic review.

HT29 and HCT-116 were the most frequently used human colon adenocarcinoma cell lines in in vitro assays studying cytotoxicity (12 assays), apoptosis, migration, and tumor hypoxia.[43,45] In fact, Corvo et al showed that encapsulation of the MG85 complex in PEGylated liposomes functionalized with Cetuximab led to 2- and 4-fold higher cytotoxicity in the HCT-116 cell line compared to free MG85.[48] Likewise, the RA-V chemotherapeutic transported together with the HIF-1a inhibitor RX-0047 in a pH-sensitive liposome targeting cell death receptor 5 (DR-5) demonstrated greater activation of the caspase-8 cascade than both compounds alone. In addition, this liposome was able to decrease protein expression of the HIF-1 factor by reducing the tumor hypoxic environment in HT-29 and/or HCT-116 cells.[45] On the other hand, HT29 tumor-bearing mice were preferred in in vivo studies. Liang et al showed that the use of immunoliposomes loaded with 5-FU in this murine model induced a significant (2-fold) regression of tumor volume and 1.5- to 1.7-fold higher cell apoptosis compared to 5-FU-loaded liposomes.[52] Furthermore, Arabi et al demonstrated a strong antitumor effect with the use of anti-CD44-

conjugated Doxil®. Specifically, at doses of 10 and 15 mg/kg, the nanoformulation increased survival time (1.4- to 1.5-fold) and reduced tumor growth by more than 94% compared to control in C-26 tumor-bearing mice.[42]

In conclusion, most of the studies showed improved therapeutic effect after the use of targeted lipid nanoformulations, except for five of them — although one of these was not studied in in vivo or in vitro assays.[53] Lipid nanoformulations appear to be the most widely used ANCs in CRC due to their favorable toxicological profile and high bioavailability.[56,57] Moreover, the possibility of adding a PEG coating to the lipid decreases its recognition by the endothelial reticular system, increasing the amount of drug available in the tumor. In fact, the use of a PEG-coated lipid nanoformulation and antibody conjugation was found in eleven articles.

Polymeric Nanoformulations

Polymeric NPs are a promising option as drug delivery systems, as they have favorable biocompatibility characteristics, good solubility profiles, and long circulation times. However, there is a translational gap between animal models and patients,[58] making them a strategy to be improved. Poly(lactic-co-glycolic acid) (PLGA), a copolymer composed of PLA and PGA, both approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA),[59] was the most frequently used polymeric NP to be functionalized with antibodies (3 out of 7 articles). Other polymers used for the synthesis of nanocarriers were poly(glycolic acid) (PGA),[60] poly(lactic acid) (PLA),[61] polycaprolactone (PCL)[62] and citrus pectin-chitosan NPs (Table 2).[63] On the other hand, 4 articles used co-polymerization with PEG to increase

circulation time, decrease immunogenicity and/or aid binding of the antibody.

All 7 articles analyzed different drugs with the exception of the studies by Fay et al and by Schmid et al, which focused on camptothecin encapsulated in PLGA-anti-DR5 and PLGA-anti TRAIL NPs, respectively.[64,65] In fact, the former led to enhanced cytotoxicity (4-fold) and increased cell apoptosis of camptothecin compared to camptothecin alone.[64] 5-FU,[61] PTX,[59] IRI,[62] carfilzomib,[66] curcumin[63] and triptolide[60] were other drugs associated with polymeric NPs. Cetuximab was used to direct nanoformulations in 2 of the 7 articles.[61,63] The most relevant result

was obtained by Sabra et al, who demonstrated that citrus pectin-chitosan NPs functionalized with curcumin-transporting Cetuximab achieved 29.8- and 30-fold higher cytotoxicity than free curcumin in CaCo-2 and HCT-116 cell lines, respectively. This targeted nanoformulation also induced 1.8-fold higher cell cycle arrest in G2/M phase compared to free curcumin in CaCo-2 cells.[63]

Finally, HCT-116 and HT29 CRC cell lines were the most commonly used in in vitro and in vivo assays. Recently, Yalikong et al assayed a triptolide delivery system based on PGA-L-Phe NPs targeted against HER2 that induced 3-fold higher cytotoxicity in HT29 cells and higher G1-S arrest compared to the drug alone.[60] In addition, 4 of the 7 articles used murine CRC models to evaluate the therapeutic efficacy of nanoformulations such as mPEG-PCL/mal-PEG-PCL NPs targeted against the CD-133 biomarker and loaded with IRI, which induced greater tumor regression in HCT-116 tumor-bearing mice compared to non-targeted NPs and free IRI.[62]

Polymeric NPs present numerous advantages that promote their application as nanovehicles in targeted therapy of CRC, being biocompatibility one of the most characteristic properties, also present in lipid nanoformulations. However, control over the shape and size of polymeric NPs allows long circulation times by evasion of the endothelial reticular system, which is associated with increased passive accumulation in the tumor via the EPR effect. Moreover, control over

design allows the development of precision chemistry for specific and orientated binding of monoclonal antibodies, which is essential for proper targeting.[67]

Inorganic Nanoformulations

As shown in Table 3, 10 articles studied the therapeutic effect of inorganic nanocarriers. Among metallic nanoformulations (8 articles), the most common were gold NPs (6 articles) followed by silica and ruthenium NPs (1 article each).[68,69] Five of them selectively targeted EGFR+ CRC cells. Notably, mifepristone-loaded mesoporous silica NPs coated with anti-EPCAM antibody were designed to selectively detect circulating tumor cells (CTC) of CRC through the membrane glycoprotein EpCAM, which is one of the most widely used surface antigens to differentiate cancer cells of epithelial origin from healthy blood cells.[70] This nanoformulation showed to decrease the adhesion of HT29 and SW620 colon cancer cells to endothelial cells by 1.3-fold. In fact, a decrease in lung metastases of SW620 tumor-bearing mice compared to the non-targeted NP and free mifepristone was demonstrated.[68] Non-metallic conformations (2 articles) used EGFR antibody; one showed the efficacy of polyethylenimine (PEI)-coated quantum dots to increase cytotoxicity in HCT-116 cells,[71] and the other analyzed the antitumor effect of nanodiamonds to inhibit tumor growth and to induce apoptosis.[74]

The drugs most frequently associated with inorganic nanoformulations were 5-FU and DOX (4 articles). Mohd-Zahid et al and Liszbinski et al functionalized gold NPs with anti-CD133 and anti-EGFR antibodies, respectively. The former enhanced the antitumor effect in the HCT-116 cell line compared to the non-targeted NP,[37] The latter demonstrated an increase in apoptotic cells compared to free 5-FU and the non-targeted nanoformulation, but no decrease in cell viability.[26] Concerning DOX, Lo et al synthesized graphene quantum dots coated with PEI polymer and functionalized with anti-EGFR that decreased DOX toxicity in a murine CRC model.[71] In addition, Emami et al developed gold NPs loaded with DOX and targeted to the immune checkpoint protein PD-L1 that enhanced in vitro antitumor effect (1.6-fold) in comparison with the non-targeted nanoformulation, and increased the number of necrotizing cells and reactive oxygen species (ROS) generation.[73] PTX,[74] OXA,[76] Zn (II) coordination compound,[72] Mifepristone,[68] RBT[69] and TS265[75] were other antitumor agents tested. The most relevant result was obtained with OXA-loaded gold NPs targeted against the DR5 receptor, a transmembrane protein belonging to the tumor necrosis factor receptor (TNFR) family, which is overexpressed in stages II and III of CRC.[77] DR5-targeted gold NPs achieved 3.2-fold higher cytotoxicity and cell apoptosis than free OXA. In vivo studies also showed greater tumor growth inhibition than both the free drug and non-targeted NP in a murine model of CRC.[76]

Therefore, gold NPs stand out among inorganic NPs as ideal candidates for the specific transport of antitumor molecules.[75] Moreover, gold NPs exhibit unique optical and Surface Plasmon Resonance (SPR) properties that are useful for tumor detection and for the development of image-based therapies, such as photothermal (PTT) and photodynamic therapy (PDT).[78] For example, DOX-conjugated and anti-PD-L1 targeting gold nanoformulations plus NIR irradiation synergistically inhibited the in vitro proliferation of the CT-26 cell line via higher apoptosis and cell cycle arrest.[73] However, unlike other types of nanovehicles, inorganic NPs present difficulties to be eliminated or excreted, resulting in adverse effects such as inflammation and tissue cysts. This limitation should be solved with the development of new biodegradable nanoformulations.[79]

Other Nanoformulations

Only 8 of the 39 selected articles analyzed hybrid (6 articles) or peptide (2 articles) nanoformulations (Table 4). Most hybrid nanoformulations were combinations of polymeric NPs and lipid nanoformulations (4 articles), usually in a polymeric micelle conformation (2 articles). In this context, Andrade et al developed CD44v6-targeted polymeric micelles loaded with niclosamide that demonstrated greater antitumor effect compared to the non-targeted nanoformulation and free drug (2.8- and 3-fold, respectively) in the HCT-116 cell line. These nanoformulations also showed activity against HT29 colonspheres and circulating tumor cells (CTC) in HT29 tumor-bearing mice.[80] Hybrid nanocarriers of inorganic metal nanocomposites (gold and silica NPs) combined with PLGA NPs and liposomes, respectively, have been also developed.[81,82] In fact, Chen et al used EGFR-targeted mesoporous silica NPs coated with PEGylated liposomes and loaded with 5-FU to increase the in vitro antitumor effect (5.8-fold), arrest in S phase (2.3-fold) and apoptosis (1.9-fold) of the drug.[81] Moreover, Ye et al developed a peptide nanoformulation using DOX-loaded fetal bovine serum (BSA) NPs functionalized with Cetuximab[83] and Agbana et al generated carfilzomib nanocarriers consisting of a polypeptide coated with PEG and targeted against the EpCAM molecule. In

this last case, cell viability assays against the DLD-1 CRC adenocarcinoma line showed a 4.5-fold increase in cytotoxicity of free 5-FU.[66]

As shown in Table 4, 5 studies focused on the chemotherapeutics 5-FU and DOX. In addition, PTX,[84] carfilzomib,[66] gamboic acid[85] and niclosamide[80] were also analyzed (each one in 1 article). For instance, Gener et al synthesized PEGylated PLGA-polymeric micelles coated with Cetuximab to transport PTX that showed a 4.4- and 16.5-fold reduction in the IC_{50} value in the HCT-8 CRC cell line compared to the non-targeted nanoformulation and free PTX, respectively,[84] Cetuximab was the most commonly used monoclonal antibody to target nanoformulations (3 articles). Anti-EpCAM,[66] the Fab fragment of anti-CD44v6 antibodies,[80] and a recombinant protein composed of an anti-EGFR antibody and the RGD peptide[85] were also used for active targeting. The HCT-116 cell line was the most widely used to assess therapeutic efficacy, followed by colonspheres. In fact, 5-FU loaded polymeric micelles functionalized against SMC2, a central component of the condensin complex involved in DNA supercoiling, demonstrated a 4-fold higher cytotoxicity in HCT-116 colonspheres, but did not show differences in antitumor effect against HCT-116 adherent cells compared to free 5-FU.[86]

Finally, although involving a more complex synthesis process compared to other nanoformulations, some hybrid NPs were developed with the aim of benefiting from the greatest possible number of advantages in a single nanocarrier. For example, Chen et al synthesized mesoporous silica NPs with an easily modifiable surface area, but they tended to form aggregates under physiological conditions, leading to low hemocompatibility. The addition of a liposomal shell allowed the generation of hybrid NPs with high biocompatibility, stability and controlled release that showed promising results in targeted therapy against CRC.[81]

Conclusion

The use of targeted nanoformulations as tumor-selective delivery systems in CRC is a promising strategy to improve antitumor efficacy. The administration of current antitumor drugs is limited by several obstacles such as rapid excretion, degradation and whole-body distribution, with the subsequent development of side effects. Regarding the latter limitation, site-specific drug delivery is necessary. An active targeting delivery system using monoclonal antibodies is an excellent way to direct pharmacological agents against tumor cells, as it induces a higher level of cell internalization compared to conventional delivery systems. In recent years, significant progress in the synthesis of high quality NPs (eg, composition, shapes, and sizes) has been made. Specifically, in CRC therapy, the NPs most frequently associated with monoclonal antibodies include polymeric NPs, lipid nanoformulations (liposomes and micelles) and inorganic NPs. In relation to the drugs most commonly used for testing antibody-functionalized nanoformulations in CRC, 5-FU and DOX were the two most outstanding chemotherapeutic agents in the studies analyzed. Furthermore, the most frequently used antibody for the generation of these nanoformulations was Cetuximab, which recognizes EGFR. Finally, a wide variety of CRC cell lines were used in in vitro assays to determine the efficacy of the newly synthesized nanopharmaceuticals, but the HT-116 cell line was by far the most commonly used. Future research is likely to include extensive development of nanoformulations with multi-ligand binding systems and exquisite specificity. However, although most of the studies included in this review yielded positive results, further assays are needed to demonstrate the benefits of novel drugs regarding bioavailability, biodegradability and biocompatibility in in vivo CRC models.

Future Perspectives

Despite recent advances in nanoformulations, including targeting systems for CRC treatment, many challenges remain to obtain a feasible alternative to conventional chemotherapy. In the case of antibody-linked nanoformulations, their specificity and efficacy still need to be greatly improved, since in many cases the tumor cells can escape the action of the nanodrug. This may be especially relevant in the case of CSCs, which show intense drug resistance and the ability to induce recurrence and metastasis. In addition, it should be noted that the toxicity of NPs may be increased by the use of antibodies. Damage to normal cells that express specific antigens recognized by the nanodrugs may limit their use. The success of this new targeted therapy against CRC will depend on the development of strategies to solve this problem. In any case, as new targeting strategies continue to be developed, more effective NP platforms will be applied in the treatment of CRC.

Acknowledgments

A.C. and F.Q. to acknowledge FPU2017 and FPU2019 grants (ref. FPU17/02977 and FPU19/04112) from the Ministerio de Educación y Formación Profesional (Spain) and C.L. a P-FIS (ref. PI20-00284) from the Ministerio de Ciencia, Innovación y Universidades (Spain). This research was funded in part by Junta de Andalucía (A-CTS-666-UGR20, P20_00540, B-CTS-122-UGR20 and PYC20-RE-035 UGR) FEDER program and Instituto de Salud Carlos III(PI19/01478-FEDER).

Disclosure

The authors declare that they have no conflicts of interest in this work.

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