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TOPIC HIGHLIGHT

2016 Colorectal Cancer: Global view

Insight to drug delivery aspects for colorectal cancer

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Abstract

Colorectal cancer (CRC) is the third most common cancer diagnosed worldwide in human beings. Surgery, chemotherapy, radiotherapy and targeted therapies are the conventional four approaches which are currently used for the treatment of CRC. The site specific delivery of chemotherapeutics to their site of action would increase effectiveness with reducing side effects. Targeted oral drug delivery systems based on polysaccharides are being investigated to target and deliver chemotherapeutic and chemopreventive agents directly to colon and rectum. Site-specific drug delivery to colon increases its concentration at the target site, and thus requires a lower dose and hence abridged side effects. Some novel therapies are also briefly discussed in article such as receptor (epidermal growth factor receptor, folate receptor, wheat germ agglutinin, VEGF receptor, hyaluronic acid receptor) based targeting therapy; colon targeted proapoptotic anticancer drug delivery system, gene therapy. Even though good treatment options are available for CRC, the ultimate therapeutic approach is to avert the incidence of CRC. It was also found that CRCs could be prevented by diet and nutrition such as calcium, vitamin D, curcumin, quercetin and fish oil supplements. Immunotherapy and vaccination are used nowadays which are showing better results against CRC.

Key words: Colorectal cancer; Receptor based targeted therapy; Gene delivery

Core tip: Colorectal cancer (CRC) is one the major worldwide health problems owing to its high prevalence and mortality rates. It is reported that over 40000 of the adult United Kingdom population are diagnosed with CRC each year. In case of early diagnosis CRC is also one of the most curable types of cancer (cure rates > 90%). This article gives an overview study of causes and etiology of colon cancer, distinct stages from stage from 0 to Ⅳ, some novel therapies are also briefly discussed in article such as receptor (epidermal growth factor receptor, folate receptor, wheat germ agglutinin, VEGF receptor, hyaluronic acid receptor) based targeting

therapy; colon targeted proapoptotic anticancer drug delivery system, gene therapy. Recent developments in screening, prevention, biomarker and genomic analysis, nutritional supplement therapy, vaccination, and chemotherapy have improved detection and significant reduction in mortality statistics.

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INTRODUCTION

Colorectal cancer (CRC)[1] is one of the leading causes of cancer related mortality worldwide, accounting more than 600000 deaths annually[2]. The CRC is the third most common cancer diagnosed in both men and women worldwide. The age, personal history, family history, racial and ethnic backgrounds are the major risk factors. Most of the CRCs occur due to lifestyle and increasing age with only a minority of cases associated with underlying genetic disorders. The CRC basically starts in the lining of the bowel and if not treated, can grow into the muscle layers underneath, and the bowel wall. There are environmental (chemicals, infectious agents, radiation) and genetic (mutations, immune system and hormone dysfunction) factors that can interact in a variety of ways to potentiate carcinogenesis[3,4]. The ultimate goal of CRC therapy is to completely eradicate the cancer cells without damaging other healthy cells of the body, and the choice of therapy depends on the state of the patient, location, and stage of the cancer.

There are many scientific and alternative methods of colon cancer treatment. Treatment trials show their higher or lower effectiveness based on stage of the cancer in the patient and the age of the patients, however outcomes for the younger patients have been good as compare to elder. However, standard-of-care treatment for the elderly patients result in equivalent long-term outcomes to those observed in the younger population; and available data support the use of aggressive surgery and adjuvant therapies in well-selected patients[5,6]. The choice of treatment method for CRC is very important because each tumor responds to different methods differentially. It is selected according to many factors including tumor type, stage of the disease, patient's age, patient's level of health, and his attitude toward life. Now a day's alternative medicine offers many methods that help a number of people. In addition, the pathogenesis of CRC, which involves a combination of many risk factors is poorly understood, and research efforts in these areas are ongoing[7].

Colon cancer: Causes and etiology

Most of the CRC cases are sporadic with no conspicuous family history or heritable tendency. Somatic mutation of the adenomatous polyposis coli (APC) is a distinctive marker of approximately 80%-85% of the patients with nonhereditary sporadic adenomatous polyposis (SAP). Mutation of the APC gene is thought to be an early step in the development of CRC, however main causes are not defined but may involve diets, smoking, environmental hazards, viruses, and life styles of different individuals (Figure 1)[8].

Few patients of colon cancer (8%-12% of all cases) are associated with heritable tendency. Genetic evidences depict that patients with familial adenomatous polyposis (FAP) inherit a germ line mutation of the *APC* gene and the might have chances of lifetime incidence of CRC. A second group of heritable CRC patients consists of those who are diagnosed with hereditary nonpolyposis colorectal cancer (HNPCC). The genetic defects in HNPCC patients are non specific but are known to be associated with the mutations of a number of DNA mismatch repair genes including MLH1, MSH2, MSH6, PMS1 and PMS2 genes[9]. A much larger third group of "heritable" CRC is those with a family history of CRC but is distinct genetically from either FAP or HNPCC cases. In addition, other signaling pathways such as epidermal growth factor receptor (EGFR)[7], the ras/raf/MAPK cascade[10], and activation of Akt kinase and STAT3 transcription factors have been implicated in the oncogenesis of CRC[11].

Classification of CRC

The CRC could be classifying mainly on the basis of histopathological features. Molecular studies have allowed a significant appreciation of the heterogeneous nature of CRC. The CRC is classified into four distinct stages.

Stage 0: Is very early stage of CRC where polyps are formed in the mucosal lining of the colon. During colonoscopy, the polyps are eradicated fully by polypectomy and prevent from development of CRC.

Stage Ⅰ: Polyp develops into a tumor and invades the inner-lining of the mucosa at this stage. Usually surgery is the main option for treating the CRC at this stage where the cancerous portion of the tissues is separated from the non-cancerous portion. Survival rate is more than 90% if CRC is diagnosed at this stage.

Stage Ⅱ: It is characterized by whether the cancer has spread beyond colon but not to the lymph nodes through metastasis. This stage is further categorized into three Stages, ⅡA, ⅡB, and ⅡC depending on the spreading of cancer to the muscular layer, or outermost layer of the colon or beyond colon[12]. Resection

surgery is the only option to menace this stage and the survivalist of the patients at this stage is more than 80%.

Stage Ⅲ: This stage of colon cancer is diagnosed with cancer has already spread all the wall of the colon and also to the surrounding lymph nodes and the survival rate is around 30%-60%. This stage of cancer is subcategorized into stage Ⅲa, b and c depending on the spreading of the cancer to the inner, middle and outer layer of colon and the surrounding lymph nodes. Along with the surgery, chemotherapy and the other medical therapy is required to treat this cancer.

Stage Ⅳ: At this stage the cancer has speeded to the other part/organ of the body like liver, ovary, testis, intestines. Survival rate is only 3%. Surgical resection, chemotherapy, radiation therapy and surgical removal of the portion of the other body parts with cancer are opted to treat at this stage of colon cancer. Colonoscopy is recommended for all 50 years or older in their routine checks[13,14].

THERAPEUTIC APPROACHES

The basic four approaches are currently used for the treatment of CRC: surgery, chemotherapy, radiotherapy and targeted therapies. The mainstay of CRC treatment is surgery. In early stage disease (stage 0 or Ⅰ), surgical excision can be used without need for further treatment options, as the recurrence rate for node-negative T1 CRC is not good enough[15]. Many studies have now shown that adjuvant therapy has a survival benefit for patients with stage Ⅲ disease, and therefore, this is the standard of care. The situation is still not very clear for stage Ⅱ CRC patients, however, in which there is somewhat conflicting, evidence regarding the benefit of adjuvant therapy. It is agreed that "high-risk" stage Ⅱ patients should be offered adjuvant therapy, as they are the most likely to derive a benefit, although there is currently some debate regarding the exact definition of "high-risk" stage Ⅱ CRC. Patients with stage Ⅳ disease require chemotherapy or targeted therapies combined with surgery[16].

Formulation based approaches for CRC

The traditional intravenous administration of chemotherapeutics for treatment of CRC, distributed drug both the desired (cancer site) and undesired (normal cells) site. However these adverse effects in undesired sites can be subsided or prevented by site-specific release of chemotherapeutic in the cancerous region of colon[17]. This would be highly beneficial in enhancing the efficacy at the site of action with no or minimal adverse effects. Drug delivery scientists explore the possibility of enhancing drug permeability through colonic epithelium which may provide local and systemic delivery of chemotherapeutics to treat cancer. A significant number of formulation and preclinical research studies have been carried out to target chemotherapeutic agents to colon *via* oral administration using this hypothesis. The colon specific drug delivery *via* oral rout have been categorized in four basic approaches: (1) temporal control of drug delivery (depends on time of passage); (2) pH-based drug delivery (triggered by a change in local pH as the formulation passes down the GIT); (3) enzyme-based drug delivery (the enzymes abundance locally in a region of the gut breakdown a prodrug or a formulation to release the active therapeutic moiety; and (4) pressure-based systems (variations in pressure along the lumen of the GI tract is used to trigger the drug release). Some other mechanism used for colon specific delivery are; osmotic controlled drug delivery, prodrug based systems, microbial triggered approach, commensal bacteria and hydrogels based systems[17,18]. All the formulation approaches having its own merits with certain limitations. To overcome the limitations associated with individual approach, and to maximize colon specific drug delivery, a permutation of two or more formulation approaches have been utilized.

The importance of carbohydrate-drug design, with a major focus on application in colon specific drug delivery has been gained interest by drug delivery researchers[19]. The vital role of natural polymers for colon-targeted delivery is based on the fact that anaerobic bacteria in the colon are able to recognize the various substrates and degrade using enzymes[18]. The colon specific delivery systems based on a single polysaccharide do not efficiently permit targeted release. The pH and transit time can vary depending on the individual patient and disease state, and drug release can be premature or even non-existent in these cases. The colon-targeted drug delivery systems reviewed herein, those based on polysaccharides, involving simple pharmaceutical preparations (*e.g.,* guar gum compression-coated tablets of 5-FU, guar gum matrix tablets of celecoxib and curcumin, solid dosage forms coated with pH- and/ or time-dependent polymers), are trouble-free in formulation development, and are considered as practicable to scale-up to a commercial product[20]. The industrial researches are going on with the use of mixtures of polysaccharide and their structurally/chemically modified forms[21-23].

The 5-FU loaded guar gum compression-coated formulations delivered higher amount of drug to colonic region with minimal amount of drug released in upper part of GIT[24,25]. After getting successful establishment of guar gum for colon-specific drug delivery of anticancer drug, other researchers have used a mixture of various polysaccharide gums (xanthan gum, boswellia gum and HPMC) as a compression coat over the core tablet for controlled release of 5-FU in colonic milieu[26]. Microspheres of 5-FU have also been designed using a pH-sensitive polymer (mixture of Eudragit® P-4135F and Eudragit® RS 100)

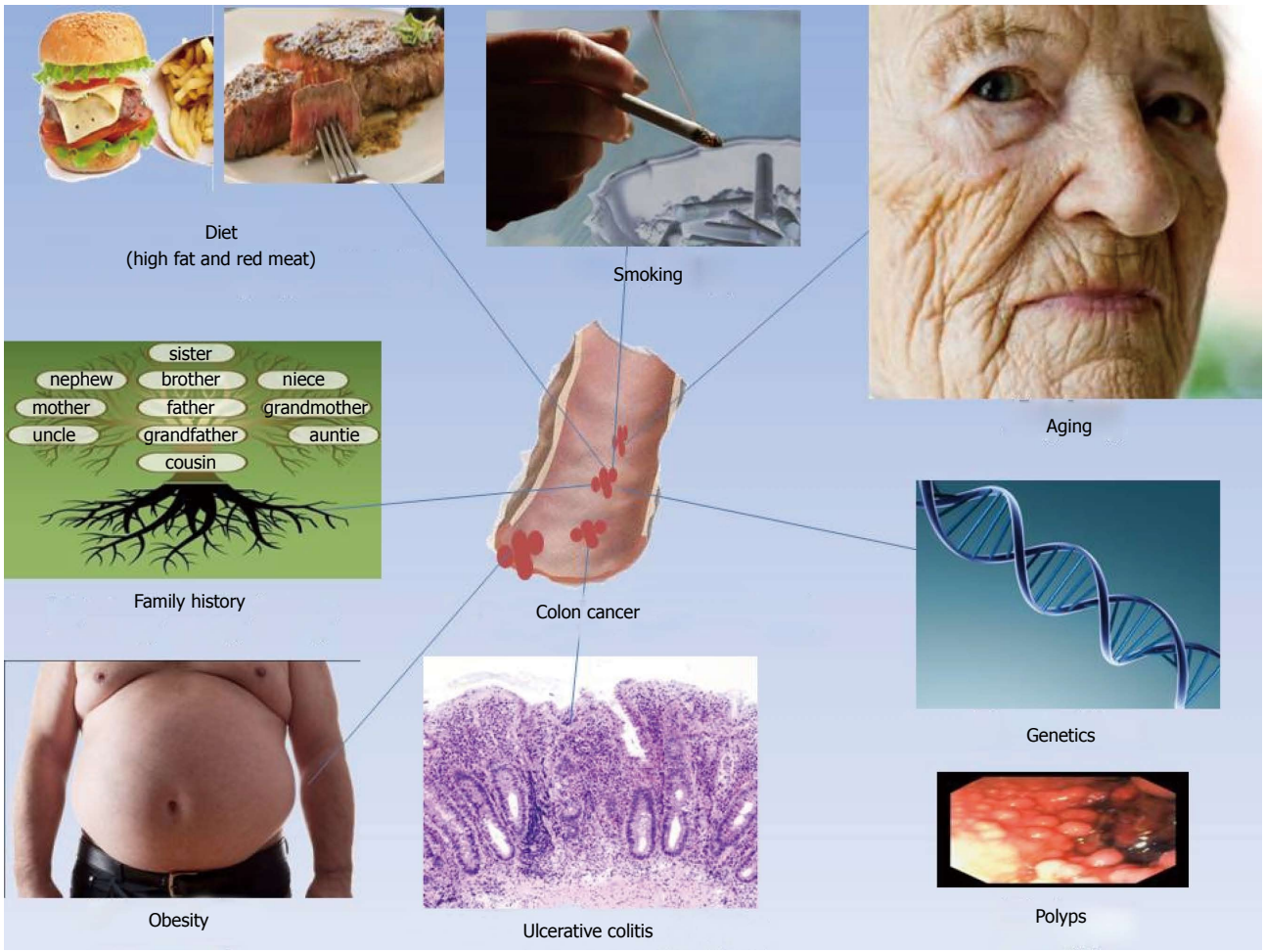

Figure 1 Basic causes of colorectal cancer.

and *in vitro* drug release studies shown the promising application for the treatment of CRC[27]. Calcium pectinate gel beads have been prepared by ionotropic gelation method followed by enteric coating with Eudragit® S100 for colon specific delivery of 5-FU[28]. The *in vitro* and *in vivo* data have suggested that orally administered 5-FU loaded Eudragit® S100 coated calcium pectinate beads effectively delivered maximum drug load to colonic site. A covalently synthesized 5-FU-pectin conjugate subjected *in vivo* distribution studies in rats and results have suggested that 5-FU-pectin conjugate is mainly distributed in cecum and colon[29]. The other researchers have developed pellets of 5-FU and film coated with various proportions of pectin and ethyl cellulose for colon-specific drug delivery[30]. The *in vitro* drug release studies have exhibited that pellets of 5-FU coated with pectin and ethyl cellulose (1:2) at a film thickness of 20% total weight gain can effectively retard or protect the drug release in upper part of GIT, yet have released 85% of the drug in simulated colonic fluids. The pellets coating thickness of 30% total weight gain have produced more sustained and satisfactory drug release profiles in the SGF, SIF and colonic fluids. The delayed Tmax, decreased Cmax and prolonged mean residence time compared with uncoated pellets have confirmed that pellets coated with thickness of 30% total weight gain can deliver 5-FU to colon for local action. Dual approach pH- and bacterially triggered system *i.e.*, Eudragit® S100 coated alginate microspheres containing 5-FU, has been utilized for treatment of CRC. The dual approach effectively delivered 5-FU to colonic tissue of the rat with less systemic side effects[31,32].

The Polysaccharides investigated for colon specific drug delivery special reference to CRC has been listed in Table 1. In addition to polymers, other drug carriers, such as Microparticles, liposomes, solid lipid nanoparticles might be used for targeting of anti-cancer drug to colon tumor (Table 1). Ogawara *et al*[33] recently investigated the effect of polyethylene glycol (PEG) liposomal doxorubicin in a male mouse tumour model inoculated with either colon cancer (C26) cells or their doxorubicin-resistant (MDR) subclone, which overexpresses P-gp efflux pumps. Over the years, various strategies have been developed to coat the liposomal surface with natural or hydrophobized polysaccharides, namely mannan, pullulan, amylopectin, dextran *etc*. or their palmitoyl or cholesteroyl derivatives[34]. The polysaccharide(s) coat tends to make these vesicular constructs physicochemically stable in

Table 1 Formulation and materials investigated for colorectal cancer

Polymer/material	Bioactives	Formulation	Remark	Ref.
Chitin	Paclitaxel	Nanoparticles	The nanoparticles were hemocompatible and *in vitro* drug release studies showed a sustained release of PTX. Anticancer activity studies proved the toxicity of PTX-AC NPs toward colon cancer cells	[35]
Chitosan derivatives	5-aminolevulinic acid (5-ALA)	Nanoparticles of methoxy poly(ethyleneglycol)-chitosan copolymer	PEG-Chito-5-ALA nanoparticles showed superior delivery capacity of 5-ALA and phototoxicity against tumor cells. These can be photodynamic therapy of colon cancer cells	[36]
Dextran	rIL-2	Dextran/PLGA-PLA core/shell microspheres	In the subcutaneous colon carcinoma BALB/c mice models, intratumorally administrated microspheres showed better local efficacy at tumor site mediated by rIL-2 from a single dose of microspheres than that of multiple rIL-2 solution injections	[37]
Pectin	5-fluorouracil (5-FU)	Zinc pectinate pellets	*In situ* intracapsular wetting of pectin coat by dissolution medium resulted in the formation of ethylcellulose plug interconnecting with pellets through the binding action of pectin. Less than 25% of drug was released at the upper gastrointestinal tract. The majority of drug was released upon prolonged dissolution and in response to colonic enzyme pectinase, which digested core pellets	[38]
Guar gum	Piroxicam	Tabletted guar gum microspheres	Crosslinked guar gum microspheres of piroxicam were directly compressed into matrix tablet and coated with Eudragit S100. The optimized tablet that displayed 0% release in simulated gastric fluid, 15% in simulated intestinal fluid and 97.1% in simulated colonic fluid underwent roentgenographic study in rabbits to check its safe transit to the colon. This could be used as targeted adjuvant therapy for colonic adenocarcinomas	[39]
Chondroitin sulphate	Indomethacin	Tablets	Tablets were prepared by cross-linking very low water soluble and relatively high water soluble polymers	[40]
Alginate	Iron-saturated bovine lactoferrin (Fe-bLf) protein	Alginate-enclosed chitosan-calcium phosphate-loaded iron-saturated bovine lactoferrin nanocarriers (Fe-bLf-loaded NCs)	After oral delivery, the pharmacokinetic and bioavailability studies indicated that nanoformulated Fe-bLf was predominantly present on tumor cells (Caco-2) compared to non-nanoformulated Fe-bLf. Fe-bLf-loaded NCs were found to help in absorption of iron	[41]
Hyaluronic acid	A near-infrared fluorescence imaging dye (Cy 5.5) and irinotecan	Poly(ethylene glycol)-conjugated hyaluronic acid nanoparticles (P-HA-NPs)	Cy5.5-P-HA-NPs and IRT-P-HA-NPs showed theranostic and therapeutic monitoring potential for colon cancer	[42]
Heparin	Plasmid-expressing mouse survivin-T34A (ms-T34A)	Heparin-polyethyleneimine (HPEI) nanoparticles	Intratumoral injection of HPEI nanoparticle-mediated ms-T34A significantly inhibited growth of subcutaneous C-26 carcinoma *in vivo* by induction of apoptosis and inhibition of angiogenesis	[43]
Pullulan	Paclitaxel	Nanoparticles of hydrophobized Pullulan (PA)	Pullulan was hydrophobically modified using acetic anhydride. The nanoparticles showed lower antitumor activity *in vitro* against HCT116 human colon carcinoma cells than that of paclitaxel itself, indicating the sustained release properties of nanoparticles. An *in vivo* study using HCT116 human colon carcinoma-bearing mice showed that paclitaxel-incorporated PA nanoparticles reduced tumor growth more than that of paclitaxel itself	[44]
Copolymer of 2-hydroxyethyl methacrylate with 4-methacryloyloxy	5-fluorouracil	Hydrogel	Drug release was faster and greater in human fecal media compared to simulated gastric and intestinal fluids. Faster release due to the cleavage of the azo crosslinks in the hydrogel by the azoreductase	[45]
pectin	Methotrexate	Microparticles (Ionotropic gelation with calcium ions)	*In vitro* drug release study showed good release of drug in presence of 3% rat caecal contents, which was further increased to more than 90% when enzyme induction was carried out for 7 d	[46]
Hydrogenated soybean phosphatidylcholine and poly ethylene glycol-2000	Doxorubicin	Liposomal coated with PEG	Investigated the effect of PEG in a male mouse tumour model and exhibited that the cytotoxic effect of DOX on vascular endothelial cells in tumor tissues would be involved in the *in vivo* anti-tumor effect of PEG liposomal DOX in P-gp over-expressing tumor-bearing mice	[33]
Triglyceride esters	5-FU	Solid lipid nanoparticles by evaporation technique	SLNs system has a high potential to improve the uptake of anticancer drugs inside colon tumors. The release profile of the drug in simulated colonic medium showed a prolonged pattern that may allow spreading of the drug inside the colon to cover most of the colonic area wherever the tumors may exist	[47]

Tablets	Meloxicam	Polyethylene oxide were dually coated with ethyl cellulose containing hydrophilic material, polyethylene glycol as an inner coating layer and Eudragit® FS 30D as outer coating	Meloxicam-loaded colon targeted system exhibited promising targeting efficacy and may be used for prophylaxis of colorectal cancer	[48]

bio-environments while retaining site-specificity[35-48].

Receptor based targeted therapy

Generally most of the drugs for CRC therapy are administered through intravenous route which showed severe systemic side-effects due to its cytotoxic effect on normal cells. Several researchers have been made significant efforts to develop novel oral formulations for colon-specific drug delivery. Treating the cancer cells with the monoclonal antibodies drugs that specifically target the cancerous cells comes under targeted therapies. These drugs have been developed over the last few years that target on signaling pathways to control cell division and tumor angiogenesis. Angiogenesis inhibitors, especially monoclonal antibody to VEGF, evacizumab, have also been developed in the last few years. Bevacizumab associated with classical cytotoxic chemotherapy led, in selected patients to an increase of median survival to more than 12 mo with tolerable toxicity. Panitumumab is a fully human monoclonal antibody directed against the EGFR and has been used in association with best supportive care for metastatic CRC treatment[49]. Combination of some of these targeted therapy with chemotherapy have shown to achieve a median survival of over 2 years[50,51].

In tumor pathology, aberrant glycosylation is a common attribute of neoplastic growth and is considered to be one of the major determinants of cancer-related phenomena such as invasive growth or metastasis. Lectins, which selectively and reversibly bind to particular carbohydrate glycoconjugate structures, have demonstrated the ability to induce and/or control a number of metabolic and proliferative processes. Therefore, lectins might be used for two functions in anticancer drug delivery systems being targets to cancer cells and inducing anticancer effect. However on the other hand, colon cancer cells overexpress certain types of lectins that might be used as targets for colon-specific drug delivery. These include the upregulation of galectins-1 and -3 in human colon cancer[51,52]. It was found that galectin-1 and galectin-3 were expressed in variable amounts in the epithelial cells and the connective tissue of normal colon. Their expression significantly increased with the degree of dysplasia, suggesting that galectin-1 and galectin-3 and their binding sites are related to malignant progression, while galectin-8 has been associated with suppressor activity. Many epithelial tumors, including colon, express both galectin-1 and galectin-3[53,54]. Therefore, these galectins can potentially be used to target colon cancer cells.

David *et al*[55] 2002 synthesized three types of N-(2-hydroxypropyl)methacrylamide copolymers containing the saccharide epitopes galactosamine (P-Gal), lactose (P-Lac), or triantennary galactose (P-TriGal). The lectin-mediated endocytosis of the HPMA glycoconjugates in human colon cancer cell lines showed potential targeting tools for cytotoxic drugs to colon adenocarcinoma. The overexpression of hyaluronan (HA) receptors on colon cancer cells might be used to enhance uptake of an anticancer drug delivery systems. The anticancer drug-polymer conjugates were developed and evaluated. A cell-targeted prodrug was developed for Taxol, using HA as the drug carrier. HA-Taxol conjugates showed selective toxicity toward the human cancer cell lines (breast, colon, and ovarian) that are known to overexpress HA receptors, while no toxicity was observed toward a mouse fibroblast cell line at the same concentrations used with the cancer cells[56]. An HPMA-HA polymeric DDS was used for targeted delivery of doxorubicin (DOX) to cancer cells. HA-DOX bioconjugates, and HPMA copolymer-DOX conjugates containing HA as a side chain (HPMA-HA-DOX) were synthesized and cytotoxicity of the polymer-drug conjugate was evaluated *via in vitro* cell culture. Study revealed that cytotoxicity of HPMA-HA-DOX targeted bioconjugate was higher against human breast cancer (HBL-100), ovarian cancer (SKOV-3), and colon cancer (HCT-116) cells when compared to the non-targeted HPMA-DOX conjugate. Fluorescence confocal microscopy revealed that the targeted HPMA-HA-DOX conjugates were internalized more efficiently by cancer cells relative to the non-targeted HPMA-DOX conjugate. Both HPMA-DOX and HPMA-HA-DOX showed minimal cyto- toxicity toward mouse fibroblast NIH 3T3 cells. Therefore, selective delivery of anticancer agents to cancer cells was achieved by biochemical targeting. The HA-modified HPMA copolymer showed improved toxicity due to receptor-mediated uptake of the macromolecular drug[57]. Jain *et al*[58] 2010, developed HA-coupled chitosan nanoparticles bearing oxaliplatin (L-OHP) encapsulated in pellets and were coated with Eudragit S100 for effective delivery to colon tumors. In therapeutic experiments the pellets of free drug, and HA-coupled and uncoupled chitosan nanoparticles bearing L-OHP were administered orally at the dose of 10 mg L-OHP/kg body weight to tumor-bearing Balb/c mice. *In vivo* data revealed that HA-coupled chitosan

Table 2 Receptor based drug targeting for the treatment of colorectal cancer

Ligand	Receptor	Formulations	Bioactives	Remarks	Ref.
Cetuximab (Ctx)	Epidermal growth factor receptor (EGFR)	Magneto-fluorescent silica nanoparticles (MFSN)	-	MFSN-Ctx produced significant MRI signal changes in a human colon cancer xenograft mouse model. The local concentration of MFSN-Ctx in a tumor was amplified by the use of an external magnetic field. MFSN-Ctx can be used for the detection of EGFR-expressing colon cancer using *in vivo* imaging approaches	[59]
Folic acid (FA)	Folate	Folic acid conjugated guar gum nanoparticles (MTX-FA-GGNP)	Methotrexate (MTX)	MTX-FA-GGNP showed better growth inhibition of Caco 2 cells due to folate receptor mediated uptake. The MTX-GGNP not only protected the release of MTX in upper gastrointestinal tract but also showed maximum release of MTX in simulated colonic fluids (pH 6.8). The *in vivo* uptake studies revealed preferential uptake of nanoparticles formulation in the colon	[60]
Wheat germ agglutinin (WGA)	WGA receptor	Wheat germ agglutinin (WGA)-functionalised chitosan-Ca-alginate (CTS-Ca-ALG) microparticles (MPs)	5-FU	MPs significantly delivered 5-FU to Caco-2 cells and increased [methyl-^{3}H]thymidine uptake. Gastrointestinal distribution was in favour of increased localization and concentration of the particles in colon region	[1]
Anti-vascular endothelial growth factor (VEGF)-antibody	VEGF receptor	Anti-VEGF antibody-conjugated dextran-coated iron oxide nanoparticles (anti-VEGF-NPs)	-	anti-VEGF-NPs demonstrated *in vivo* tumor targeting and efficient accumulation in tumor tissues after systemic delivery in a colon cancer model	[61]
Hyaluronic acid (HA)	HA receptor, CD44	HA modified mesoporous silica nanoparticles (HA-MSNs)	Doxorubicin hydrochloride (Dox)	Dox loaded HA-MSNs showed greater cytotoxicity to HCT-116 cells than free Dox and Dox-MSNs due to the enhanced cell internalization behavior of HA-MSNs.	[62]

nanoparticles delivered higher amount of drug towards tumor site, reflecting targeting potential to the colonic tumor. These drug delivery systems show relatively high local drug concentration in the colonic milieu and colonic tumors with prolonged exposure time, which enhances antitumor efficacy with low systemic toxicity for the treatment of colon cancer. Colon-specific HPMA copolymer-9-aminocamptothecin conjugates have been designed for potential treatment of CRC[59]. 9-AC has been attached *via* spacers containing amino acid residues and aromatic azo bonds. The results of the *in vitro* drug release studies have indicated that conjugates containing leucylalanine showed high colon-specific release of 9-AC when compared to alanine containing conjugates. Some examples of receptor based drug targeting for the treatment of CRC have been tabulated in Table 2 and approaches are represented in Figure 2[1,59-62].

Colon targeted proapoptotic anticancer drug delivery system: In addition to undesirable side effects, the efficacy of colon cancer chemotherapy is limited by the activation of antiapoptotic cellular defense mechanism during adaptation to the treatment. It was well reported that acute and chronic exposure of different cancer cells to chemotherapy led to the overexpression of proteins responsible for cellular antiapoptotic defense which ultimately increased resistance of cancer cells to chemotherapy[63,64]. This type of cellular resistance to chemotherapy is known as "non-pump resistance" and distinguish from the "pump resistance", which depends on the over expression of drug efflux pumps[65]. The up-regulation of cellular antiapoptotic system plays the vital role in line of defense and BCL-2 family proteins are very important in this system[66,67]. Although overexpression of BCL-2 protein does not interfere with the entry and accumulation of drugs in tumor cells. The BCL-2 protein family consists of two types of proteins with counter modulating functions: (1) the group that suppresses apoptosis if overexpressed; and (2) the group that has the ability to induce apoptosis[66-69]. Although the precise role of these proteins in apoptosis induction and development of resistance during cancer therapy remains unclear, it was found that the expression ratio of antiapoptotic members of BCL-2 protein family to proapoptotic members determines survival or death following an apoptotic stimulus after chemotherapy of cancer cells[68]. It is logical to hypothesize that a suppression of antiapoptotic and/or activation of proapoptotic members of BCL-2 protein family could significantly induce apoptosis, and therefore, increase the efficacy of therapy. The BCL-2 family is characterized by specific regions of homology termed BCL-2 homology (BH1, BH2, BH3, BH4) domains and are critical to the functions of these proteins, including their impact on cell survival and their ability to interact with other family members and regulatory proteins[70]. It was found that the BH3 domain of proapoptotic proteins from the BCL-2 family is responsible for the induction of apoptosis[71,72]. Moreover, it was found that short synthetic peptides, corresponding to the minimal sequence of BH3 domain when bound to the antiapoptotic BCL-2 family proteins, suppress the cellular antiapoptotic defense[73].

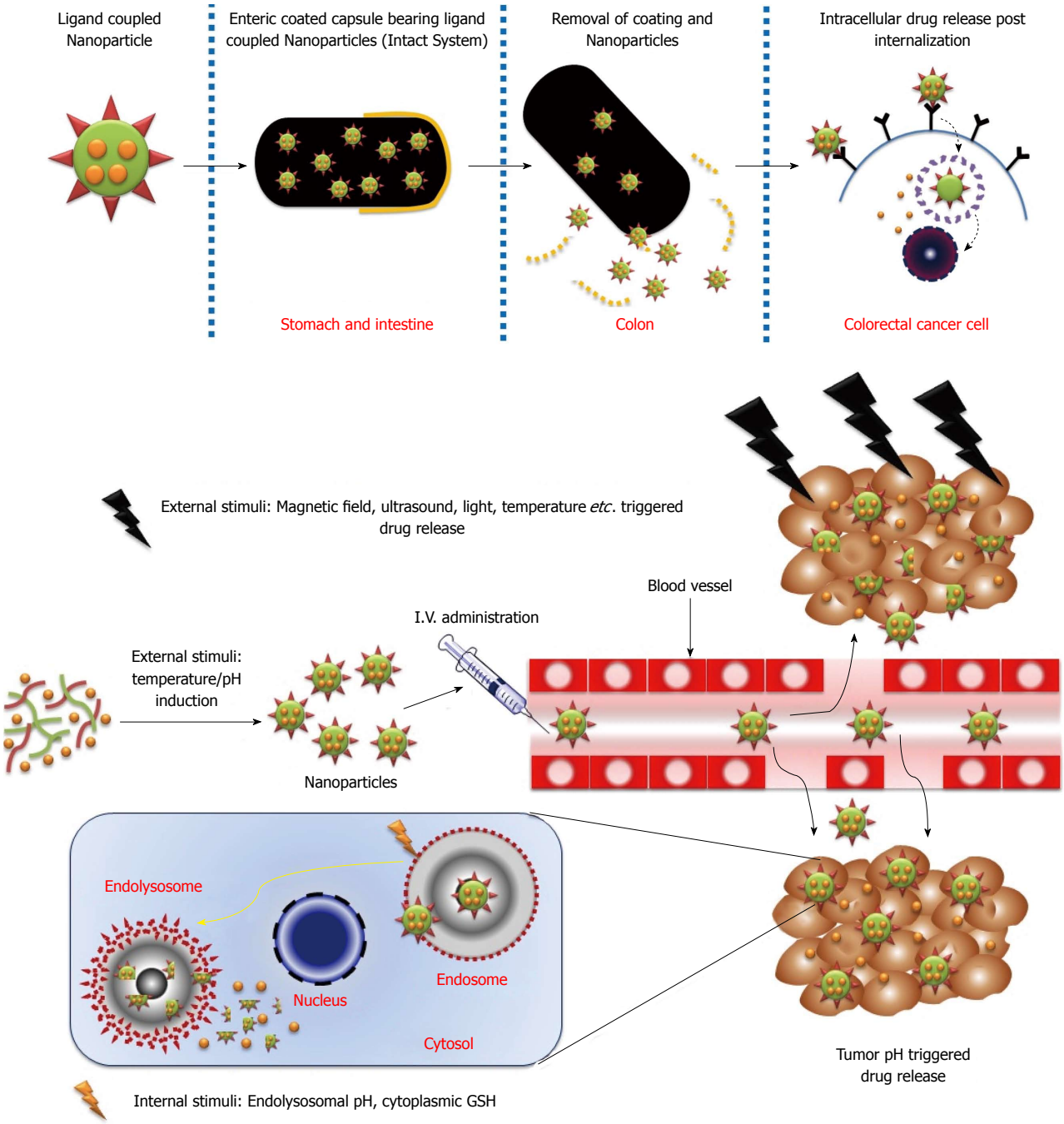

Figure 2 Schematic representation of different targeting approaches for colorectal cancer.

Therefore, BH3 peptide can potentially improve traditional cancer therapy by decreasing the resistance of cancer cells to chemotherapeutic agents. Colloidal nanoparticle could able for selective delivery of the bioactive to the disease site and can be enhanced by coating the surface of the nanoparticles with targeting moieties. Fay *et al*[74] developed PLGA nanoparticles coated with Conatumumab (AMG 655) death receptor 5-specific antibodies (DR5-NP) for the induction of apoptosis in colorectal HCT116 cancer cells and exhibited DR5-NP preferentially target DR5-expressing cells with sufficient density of antibody paratopes to induce apoptosis *via* DR5, unlike free AMG 655 or non-targeted control nanoparticles. They also demonstrate that DR5-targeted nanoparticles encapsulating the camptothecin are effectively targeted to the tumour cells, thereby producing enhanced cytotoxic effects through simultaneous drug delivery and apoptosis induction. Schmid *et al*[75] developed and optimized polymer-based nanotherapeutic incorporating both a functionalized PEG layer and targeting antibodies to limit premature phagocytic clearance whilst enabling targeting of DR5-expressing tumor cells. The HCT116 CRC model was used and showed that following binding to DR5, the nanoparticles activate caspase 8, enhancing the anti-tumor activity of the camptothecin payload both

in vitro and *in vivo*. The combination of nanoparticle-induced DR5 clustering with camptothecin delivery overcomes resistance to DR5-induced apoptosis caused by loss of BAX or overexpression of anti-apoptotic FLIP. Li *et al*[76] compared the efficacy of liposomal curcumin with oxaliplatin, a standard chemotherapy for this malignancy. *In vitro* treatment with liposomal curcumin induced a dose-dependent growth inhibition and apoptosis [poly(ADP-ribose) polymerase] in LoVo and Colo205 cells. *In vivo*, tumor growth inhibition was also observed in Colo205 and LoVo xenografts, and the growth inhibition by liposomal curcumin was higher than that for oxaliplatin in Colo205 cells. Tumors from animals treated with liposomal curcumin showed an antiangiogenic effect, including attenuation of CD31 (an endothelial marker), vascular endothelial growth factor, and interleukin (IL)-8 expression by immunohistochemistry. This study establishes the comparable or greater growth-inhibitory and apoptotic effects of liposomal curcumin with oxaliplatin both *in vitro* and *in vivo* in CRC. The main aim of site specific drug delivery to colon is to protect the drug from degradation in stomach and small intestine, to release maximum drug load to the colonic region. The benefits of colon specific delivery include lower dose and minimizing the side effects.

Gene therapy

Now a day Gene therapy considered as a potential novel treatment for CRC. In this regards the preclinical data are hopeful and some clinical trials are ongoing for CRC. To refine the approach of gene therapy, continuing efforts should be made to improve the antitumour potency, efficiency of gene delivery, and accuracy of gene targeting. The gene therapy will be incorporated into already existing therapies such as surgery, radiotherapy and chemotherapy. Gene therapy has been explored as a possible option for cancer treatment. Till date, 60% of over 600 gene therapy clinical trial protocols have been activated which pertain to cancer gene therapy in the United States[77]. Similarly in the United Kingdom, of about 50 gene therapy protocols are related to cancer gene therapy[78]. Mainly these trials have been in phase Ⅰ dose finding/safety/toxicity studies, among them 1% being phase Ⅲ randomized studies against current best practice. Specific targeting of the tumour site is the hypothetical benefit of gene therapy approaches for cancer treatment, which can reduce systemic toxicity of conventional drug therapy due to the accuracy of gene delivery and expression at the specific targeted site(s). Both prerequisites are in fact obstacles to its success. However, the efforts in developing novel gene delivery vectors, in discovering new therapeutic genes and in exploring tumour biology in order to overcome the hurdles, are currently making this conceptually new field of medicine potentially promising[79].

Immune stimulation: The purpose of immune stimulation (immunogene therapy) is to activate a systemic and tumour-specific immune response, which may be either cell-mediated or antibody-dependent, against the tumour cells.

Utilisation of human leukocyte antigen to stimulate T-cell response: Human leukocyte antigen (HLA) class Ⅰ molecules are down-regulated in up to 60% of CRCs. Animal studies have demonstrated that the expression of foreign major histocompatibility complex (MHC-the analogue of HLA in humans) on tumours can induce a T-cell dependent anti-tumour immune response. Gene transfer of HLA-B7 has been also examined in clinical trials, and HLA-B7 gene transfer has shown the ability to elicit systemic antitumour immune response in humans. Yet, no local antitumour effect was shown in hepatic colorectal metastases as has been demonstrated in melanomas.

Utilization of cytokines to stimulate T-cell response: Cytokines play an important role in co-ordinating the immune response. Therefore, the insertion of genes encoding cytokines presents a potential strategy to increase the immunogenicity of tumours and overcome immune tolerance. Pre-clinical models have tested a range of cytokines including IL-2, IL-4, IL-12, granulocyte macrophage-colony stimulating factor (GM-CSF), and interferon-γ (IFN-γ). Gene therapy with IL-2 has shown antitumour efficacy in a murine model of CRC and has been investigated in patients with CRCs[80]. *Ex vivo* IL-2 transduction to autologous fibroblast trial shows it is possible to induce a cellular immune response using IL-2 gene therapy in patients with CRC safely and with minimal toxicity. However, no objective responses were demonstrated[81,82]. *Ex vivo* IL-4 transduction to autologous fibroblasts: A phase Ⅰ study has been undertaken in which patients with advanced cancer were vaccinated with a combination of IL-4-transduced fibroblasts plus autologous irradiated tumour cells. Tissue biopsies of vaccination sites revealed that IL-4 mRNA could be detected by RT-PCR even 2 wk after initial vaccination, confirming the effectiveness of this strategy in generating IL-4 *in vivo*. However, induction of an antitumour immune response was not reported[83].

***Ex vivo* IL-2 transduction to autologous immune effector cells:** Another approach has been to transfect autologous immune effector cells with the IL-2 gene. Preclinical studies have shown that cytokine-induced killer cells. There was evidence of biological activity as indicated by an increase in serum IFN-γ, GM-CSF and TGF-β during treatment and also an increase in the cytotoxic activity of circulating lymphocytes tested against a range of HLA-matched carcinoma cell lines. Three patients were reported to demonstrate

disease stabilisation following treatment. One patient with follicular B-cell lymphoma achieved a complete response[84].

IL-2 transduction using other vectors: Two further phase I studies treating patients with a range of advanced cancers have utilized either allogeneic fibroblasts secreting IL-2, or an IL-2 DNA/lipid complex delivered by direct intratumoural injection[85,86]. Both approaches were well-tolerated with evidence of biological activity (detection of IL-2 on tumour biopsy and tumour infiltration by T-cells) *in vivo* as well as clinical objective responses in some patients (with melanoma or renal carcinoma).

So it can be consider that cytokine gene therapy is safe when mediated *via* a number of different vectors including subcutaneous injection of retrovirus-transduced fibroblasts with autologous tumour cells, cytokine-transduced immune effector cells, and direct intratumoural injection of cationic lipid vectors. So, co-expression of cytokines with, say, HLA-B7 may augment antitumour immune response and may be a useful therapeutic strategy. Now, preclinical studies using a replication-deficient adenovirus vector to deliver IL-2 suggest that the adenoviral vector itself may enhance cytotoxic T lymphocytes (CTL) recognition of tumour antigens, implying that such a vector may have therapeutic advantage in cytokine-mediated immunotherapy[87].

Carcino-embryonic antigen: Carcino-embryonic antigen (CEA) is a cell-surface glycoprotein over-expressed on the majority of CRC cells, and is only expressed at low levels in normal colon and biliary epithelium. On the basis of differential expression levels vectors are as follows:

Vaccinia vector: The immunization of CEA transgenic mice with recombinant vaccinia virus containing the CEA gene induced anti-CEA antibodies and cell-mediated immune response against subsequent challenges with CEA-expressing tumours[88]. Several phase I trials have currently tested recombinant vaccinia vectors encoding full-length CEA administered subcutaneously or intradermally at doses between 10^7-10^8 PFU to patients with metastatic CRC. These studies confirm this to be a well-tolerated treatment with toxicity confined to low-grade fever, fatigue and inflammation at the injection site[81,89]. Anti-CEA antibodies could also be detected and, although these were of low resemblance and greed, it is still the first demonstration that such antibodies could be generated in response to a recombinant vaccinia virus[80].

Canary pox vector: A important trouble with vaccinia vectors is the generation of neutralising antibodies, which may limit efficacy. This has lead to the use of canary pox vectors. This virus is not pathogenic in humans and does not reproduce in human cells. It may, therefore, be given repeatedly without neutralisation by antibodies. A recombinant canarypox virus containing the human CEA gene has demonstrated antitumour efficacy in mice[81].

Designer T-cell: A novel approach to generating an immune response to CEA is the development of anti-CEA "designer T-cells".

Mutant gene correction: Gene correction is a logical approach in cancer therapy based on the understanding of the carcinogenic processes at the molecular level. However, for most cancers, including CRC, this goal is elusive because malignant transformation is usually accompanied by an accumulation of genetic mutations, as well as clonal heterogeneity. Furthermore, the success of this approach very much relies on a high efficiency of gene expression at the tumour site(s) in order to achieve therapeutic benefit. However, the phenotypic correction of key genetic aberrations of malignant cells has shown the potential to trigger induction of apoptosis in preclinical studies. Different strategies including tumour suppressor gene correction (*e.g.,* p53) or oncogene suppression (*e.g.,* K-ras) have shown antitumoural effect in animal models of CRC and p53 gene correction delivered in adenoviral vectors is being tested in clinical trials in combination with conventional chemotherapy.

p53 tumour suppressor gene correction: About 50% of CRCs port p53 mutations. It has been shown in an animal model that re-expression of wild-type p53 in p53-mutated colon cancer xenografts can lead to an inhibition of tumour growth and increased animal survival[90]. An additional bonus for wild-type p53 correction is its by-stander effect, which is thought to be due to the anti-angiogenic effect in tumours with p53 mutation[91]. Many clinical trials using a replication-deficient adenoviral vector to deliver wild-type p53 to a range of human tumors have been carried out. Initial studies demonstrated the safety of direct intratumoural injection of these vectors and confirmed p53 gene expression even in the presence of an anti-adenovirus immune response. Another group have examined the safety, tolerability and pharmacokinetics of a replication-deficient adenovirus p53 vector (RPR/INGN-201) given intravenously to patients with advanced cancer. Five patients were treated at the maximum dose of 1-10^{12} virus particles, one of them developed dose-limiting toxicity in the way of grade 3 diarrhoea. Other toxicity was mild, and repeated monthly administration was possible, with one patient with advanced CRC receiving 10 cycles safely. Data pertaining to intratumoural p53 expression are pending[92].

More gene correction strategies: Many colorectal tumours are positive for microsatellite instability (MSI^+). Such tumours commonly have mutation in

the retinoblastoma zinc finger gene, RIZ1, but rarely have *p53* mutations. In mouse xenograft models, such tumours do not respond to adenovirus-delivered *p53* gene therapy whereas adenovirus-delivered RIZ1 can suppress tumour growth and induce apoptosis, suggesting that this may be a useful therapy for MSI^+ CRC[93].

Nutritional supplement therapy

It is estimated by Health experts that about 70 percent of CRCs could be prevented by diet and nutrition.

Calcium and vitamin D: Those who consume relatively high levels of Calcium and vitamin D supplements seem to be protected to some degree against colon cancer. Dietary factors are considered to be important in its risk in case of colon cancer which is one of the most commonly diagnosed cancers worldwide. Calcium supplements can protect against colon polyps, mainly the advanced type that go on to become cancer. Calcium and α-tocopherol suppress chemically induced colon carcinogenesis in rats and reduce associated biomarkers in human volunteers[94].

Curcumin and Quercetin: Curcumin, which is found in the curry spice turmeric, may be of benefit for patients with pre-cancerous polyps in the colon and quercetin, an antioxidant found in onions, experienced a marked reduction in both the size and number of polyps. The potential of curcumin to prevent and/or treat cancer in the lower intestines surfaced in studies in lab rats fed curry, as well as in observational studies of Asian populations that consume a lot of curry. Quercetin has also been shown to have anti-cancer potential. In their study, Cruz-Correa *et al*[95] gave five FAP patients who had five or more polyps in their lower intestinal tract with 480 milligrams of curcumin and 20 milligrams of quercetin three times daily. All five patients had a decreased polyps number and size from baseline after a mean of 6 mo. They found that average number of polyps dropped by 60 percent, and the average size dropped by 51 percent, with minimal side effects. Of the two compounds, the researchers believe curcumin is the key cancer-fighting agent. The amount of quercetin administered was similar to what many people consume daily; however, the amount of curcumin is many times what a person might ingest in a typical diet[95]. Guar gum matrix tablets of curcumin has been developed for colon-specific delivery to its possible use in the prevention of CRC[96]. The microbial triggered drug release from guar gum matrix tablets has been assessed in the presence of rat cecal contents. The results have shown that a 40% guar gum containing formulation can be used to deliver curcumin to colon for possible colon cancer treatment.

Fish oil supplements: Fish oil may be beneficial for cancer patients. People who eat plenty of fish oil and other omega-3 fatty acids could cut their risk of colon cancer[97].

Immunotherapy

Immunotherapy is one of most modern method for colon cancer treatment. Approaches using antibodies and vaccines as adjuvant therapy are being studied by many researchers. Cancer immunotherapy is a promising and effective treatment modality for patients with cancers. Cytokine, anticytokine, and antibody therapies appear to be efficient for treatment of various forms of cancer. Clinical trials have demonstrated that adoptive cell therapy using tumor-infiltrating lymphocytes can induce tumor regression in approximately 75% of metastatic melanoma patients, moreover, genetically engineered T cells transduced with genes encoding specific T cell receptors and chimeric antigen receptors have been shown effective in the treatment of cancer patients. These studies suggest that combination therapies are superior choices in cancer immunotherapy for patients[98].

Tumor-associated antigens (TAA) which is able to activate the immune system can express on colon tumor cells, mAbs directed against tumor antigens can opsonize tumor cells and support their elimination either by activation of cellular immune effectors like NK cells or by activation of the complement cascade. Edrecolomab (monoclonal antibody 17-1A), a murine monoclonal antibody that recognizes the epithelial cell-adhension molecule known as Ep-CAM, appears to be highly effective in treating micro-metastatic disease[99]. A study done by Riethmüller *et al*[100] on 189 patients with resected stage Ⅲ CRC, treatment with edrecolomab resulted in a significant increase in the overall survival rate, and a decrease in the tumor recurrence rate of 23%. But mAb may also clear circulating tumor cells that have greater access to immune effector cells, and is unsuccessful against cancer cells already residing within solid tissues. To increase the anti-tumor efficacy of mAb, Bi-specific antibodies have been engineered. Anti-CD3/CEA or anti-CD3/Ep-CAM bi-specific antibodies can cross-link T cells to colon cancer cells. Furthermore, anti-CEA /B7 can convert B7-negative tumors into B7-positive tumors, afford both functional and tumor-specific signals 1 and 2 for the activation of primary human T cells, and trigger their cytotoxic activity in a tumor-specific way[101,102]. The effect is limited, however, it can be improved by increasing immunogenicity of whole-cell vaccines based on the use of nonspecific immunostimulants[103]. A recent phase Ⅲ clinical trial in colon cancer with autologous tumor cell-BCG vaccine demonstrated that OncoVAX® in an adjuvant setting significantly prolongs recurrence-free interval (57.1% relative risk reduction) and improves 5-year overall survival and recurrence-free survival in Stage Ⅱ colon cancer patients. No significant prognostic benefits were achieved in Stage Ⅲ patients[104].

Improvement in host immunity against tumor cells can be achieved by promoting the differentiation of dendritic cells (DCs) from immature myeloid cells that accumulate in the bone marrow and lymphoid organs was also reported. There are some researchers are trying to target DC *in vitro* with tumor-derived RNA, while others are trying to load other recombinant TAA[105,106]. Vaccination of antigen presenting cells can present endogenous tumor antigens, activate CTL, and well induce specific anti-tumor immunity. Endothelium can also serve as a vaccine, and induce an autoimmune response targeting tumor angiogenesis. Okaji *et al*[107] immunized BALB/c mice with a vaccine of glutaraldehyde-fixed murine hepatic sinusoidal endothelial cells (HSEs) in a lung metastasis model of Colon-26 cancer. Vaccination with autologous HSEs induced both preventive and therapeutic antitumor immunity, which significantly inhibited the development of metastases. The T cell is the effector cell in antitumor immune response. T cells require 2 distinct signals for optimal activation, and the genetic modification of T cells *ex vivo* and their reinfusion into cancer patients has recently attracted considerable attention. Recent studies revealed that gene-engineered T cells expressing chimeric single-chain (scFv) receptors were capable of co-delivering CD28 costimulation and T cell receptor zeta chain (TCR-zeta) activation signals, which after reacting with the ErbB2 TAA of colon cancer produced high levels of cytokines, proliferated vigorously, and mediated lysis of ErbB2(+) tumors in an antigen-specific manner. Most importantly, dual specific T cell delayed the growth of subcutaneous ErbB2(+) human tumors after systemic administration[108].

Interactions between cancer and the immune system: The immune system, including innate immune system and adaptive immune system, is composed of special factors, cells, tissues, and organs. It has been well known that the immune system can detect a wide variety of infectious organisms and other invaders, differentiate them from the own healthy cells and tissues, and prevent infections[109].

It has been reported that there is interaction between the immune system and cancer as evidenced by cancer immunosurveillance theory which refers to the monitoring function of the immune system in cancer development. There are various studies that have demonstrated that the immune system can recognize and eliminate abnormal cancer cells arising within the human body[110-115]. Therefore, the interaction between cancer and the immune system plays a crucial role in cancer development. In cancer patients, the immune system is not sufficiently vigorous to eliminate cancer cells, suggesting that the antitumor immune system is suppressed. For instance, transplant recipients under continued immunosuppression displayed a significantly higher risk of developing *de novo* tumors such as lung cancer[116] and noticeably increased threat for cervical cancer in immunosuppressed women[117].

It has been shown that several factors may contribute to antitumor immunosuppression, for instance, a low frequency of high-avidity antitumor T cells, presence of $CD4^+CD25^+$ regulatory T (Treg) cells, and various strategies of cellular-mediated tumor-induced immune evasion[118]. Antitumor immunosuppression may also result from soluble factors and altered antigenicity[119]. For instance, tumor-derived IL-18 induced immunosuppression in NK cell-controlled cancers[120,121]. IL-1α upregulated TGF-beta in mesenchymal stem cells and thus induced immunosuppression, foremost to the growth of prostate cancer cells[122]. Hence, immunological methods that eliminate antitumor immunosuppression and/or increase antitumor immunity can be very useful in the treatment of cancer.

Cytokine and anticytokine therapy

Cytokines are group of proteins, including IL and growth factors, which play a vital role in the regulation of the immune system[123]. Several studies have indicated that cytokine therapy could relieve immunosuppression in cancer patients[124] and therefore, cytokine therapy has the potential use in cancer treatment. IFN-α was the first cytokine, which was approved and used for leukemia[125,126] and melanoma patients[127,128]. IFN-α therapy turned out to be an effective method for the treatment of leukemia and melanoma. Now a day, IFN-α therapy is the preferred choice for the treatment of metastatic melanoma[129]. There are lots of efforts to develop the efficacy of IFN-α therapy for cancer treatment. At the end, efforts are to develop a combination therapy, a combination of IFN-α therapy with other treatment modalities such as chemotherapy that can be more effective in the treatment of cancer[130].

IL-2 was the second cytokine in 1998, when United States Food and Drug Administration (FDA) approved (IL-2) therapy for cancer treatment. Clinical trials confirmed that IL-2 therapy was effective in the treatment of patients with solid tumors such as metastatic melanoma and renal cell carcinoma[131]. The combination therapies of IL-2 with other cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF)[132] or the sequence-specific combination of IL-2 therapy with chemotherapy such as temozolomide[133] appeared to be more beneficial in cancer treatment.

Tumor necrosis factor (TNF)-α has also been exposed for antitumor activity. TNF-α was used to treat patients with advanced solid tumors. Though, the use and efficacy of TNF-α therapy for cancer treatment are notorious[134]. Modern studies showed that there was an association between the anti-TNF treatments such as adalimumab for noncancer patients and incidence of malignancies such as melanoma[135]. Additionally, TNF-α administration induces serious side effects such as septic shock-like condition, which strictly restricted

TNF-α therapy[136]. GM-CSF is a cytokine created by immune cells (including T cells, NK cells, macrophages, and mast cells), endothelial cells, and fibroblasts. GM-CSF stimulates white blood cells to grow and stem cells to differentiate between granulocytes and monocytes. Clinical trials have indicated that GM-CSF-stimulating factor-secreting cancer immunotherapy in combination with primed lymphocytes and autologous stem cell transplantation in hematologic malignancies was potentially effective against acute myeloid leukemia[137]. In addition, a combination therapy of GM-CSF and IL-2 showed more benefits for melanoma patients. The results indicated that GM-CSF is useful tool as cytokine therapy for cancer patients[138].

Cytokines play an important role in the regulation of immune responses. However, its therapy rarely results in complete cure for cancer patients due to its indirect antitumor activity. On the other hand, Cytokine administration has induced toxicities in patients, which limits its potential for cancer therapy. Cytokine therapy may be effective for cancer treatment if the high concentration of cytokines is achieved at tumor sites. In recent years, targeted delivery of cytokines has become possible and indeed antibodycytokine fusion proteins (immunocytokines) seem to be effective in management of cancer treatment. Earlier Studies in mouse tumor models have shown that the antibody-mediated targeted delivery of IL-12 was found to be very effective in cancer treatment, indicating that immunocytokine therapy shows potential in cancer treatment[139,140].

Some cytokines have been shown to cause immunosuppression in cancer patients and so have the potential to be used as anticytokine therapy for cancer treatment. There are few reports that IL-6 is a vital tumor promoter in early colitis-associated cancer. Tumor-infiltrating myeloid cells produces IL-6, increases cancer development in the intestinal epithelium and protects cancer cells from apoptotic eradication. Thus, anti-IL-6 therapy may be used in the treatment of CRC[141]. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) binds to the B7-family proteins, resulting in the suppression of T cell functions. Treatment with the CTLA-4 antibodies (ipilimumab and tremelimumab) has been widely used in metastatic melanoma and showed good benefits with prolonged survival[142]. Clinical trials discovered that anti-PD-1 antibody (BMS-936558) and anti-PD-L1 antibody (BMS-936559) were safe and useful for the treatment of patients with melanoma[143]. Therefore, anti-CTLA-4, anti-PD-1, and anti-PD-L1 antibodies may effectively be used in the treatment of patients with melanoma.

VACCINATION

The human papillomavirus (HPV) vaccine is protective for cervical cancer, and this discovery has covered the way to the development of cancer vaccines for other forms of virus-associated cancers such as liver cancer and Merkel cell carcinoma. Cancer vaccines are prepared to elicit an immune response against tumor-specific or TAA, encouraging the immune system to attack cancer cells bearing these antigens. Tumor antigens that have been targeted in CRC include CEA, MUC1, guanylyl cyclase C, and NY-ESO-1. Cancer vaccines are being developed to treat patients with existing cancer and are one of the most active fields in cancer research[144]. Treatment with cancer vaccines, an active immunotherapy for cancer, is a desired method for cancer patients, especially patients with cervical cancer as cervical cancer is caused by viruses, such as, HPV. Thus, similar to traditional vaccines against viruses, vaccines are being developed to treat such types of cancer, for example, Cervarix[145] and Gardasil[146].

Sipuleucel-T, which elicited an immune response to prostatic acid phosphatase expressed on prostate cancer cells, was the first FDA-approved therapeutic cancer vaccine for patients with metastatic prostate cancer[147]. Clinical trials showed that the therapeutic telomerase- (TERT-) specific vaccine Vx-001 could induce TERT-reactive T cells and have a noteworthy survival benefit in patients with chemoresistant advanced solid tumors[148]. However, there are currently no therapeutic vaccines available to treat cancers effectively. It has been reported that the synthetic vaccine of HPV E7 protein-derived peptide antigen and star polymer could be used to treat HPV-related cancers in mice[149]. As an effective therapeutic cancer vaccine candidate, the live attenuated vaccine strain (oncolytic measles virus, MV) engineered with GM-CSF gene has been shown to induce a complete tumor regression and rejected following tumor reengraftment in murine colon adenocarcinoma model[150,151]. Furthermore, both GM-CSF and TNF-α-modified RM-1 prostatic cancer cell vaccine, which was superior to single GM-CSF- or TNF-α-modified vaccine, significantly prolonged the survival in the mouse model[152]. Therefore, the effectiveness of these conjugated cancer vaccines in mouse tumor models provided evidence for future clinical trials.

It has been well identified that cancers result from multiple gene mutations, which are diverse among individual cancer patients. Therefore, antitumor immune responses induced by TAA are different from one cancer patient to another[153], suggesting that single cancer vaccine may not be effective for all of the cancer patients. Personalized cancer vaccinations may provide a future direction for cancer treatment as therapeutic cancer vaccines. Besides, combination therapy with cancer vaccines and other conventional treatments such as radiation therapy may result in significant tumor regression and serve as a potential treatment regimen for cancer patients[154].

CONCLUSION

CRC is one the major worldwide health problems owing to its high prevalence and mortality rates. It is

reported that over 40000 of the adult United Kingdom population are diagnosed with CRC each year. In case of early diagnosis CRC is also one of the most curable types of cancer (cure rates > 90%). However, increased understanding of the molecular mechanisms underlying carcinogenesis has spurred focus on the development and incorporation of molecular targeted agents in current therapeutic options for CRC. Recent developments in screening, prevention, biomarker and genomic analysis, nutritional supplement therapy, vaccination, and chemotherapy have improved detection and significant reduction in mortality statistics. However, despite these advances, many patients with advanced and metastatic tumors will still succumb to the disease. Further research to explain the molecular pathology of CRC may improve treatment options and the long term survival of patients.

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