Novel targeting approaches and signaling pathways of colorectal cancer: An insight

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Author contributions: Tiwari A drafted the manuscript after searching appropriate literature; Panda PK contributed in the writing work; Saraf S and Verma A contributed the related figures; Jain SK critically interpreted the findings of other scientists and revised the final version of the manuscript.

Conflict-of-interest statement: No conflict of interest exists.

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Manuscript source: Invited manuscript

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Received: July 10, 2018
Peer-review started: July 10, 2018
First decision: July 18, 2018
Revised: August 24, 2018
Accepted: October 5, 2018
Article in press: October 5, 2018

Published online: October 21, 2018

Abstract

Colorectal cancer (CRC) is the third most common cancer of mortality in the world. Chemotherapy based treatment leads to innumerable side effects as it delivers the anticancer drug to both normal cells besides cancer cells. Sonic Hedgehog (SHH), Wnt wingless-type mouse mammary tumor virus/β-catenin, transforming growth factor-β/SMAD, epidermal growth factor receptor and Notch are the main signaling pathways involved in the progression of CRC. Targeted therapies necessitate information regarding the particular aberrant pathways. Advancements in gene therapies have resulted in the recognition of novel therapeutic targets related with these signal-transduction cascades. CRC is a step-wise process where mutations occur over the time and activation of oncogenes and deactivation of tissue suppressor genes takes place. Genetic changes which are responsible for the induction of carcinogenesis include loss of heterozygosity in tumor suppressor genes such as adenomatous polyposis coli, mutation or deletion of genes like p53 and K-ras. Therefore, many gene-therapy approaches like gene correction, virus-directed enzyme-prodrug therapy, immunogenetic manipulation and virotherapy are currently being explored. Development of novel strategies for the safe and effective delivery of drugs to the cancerous site is the need of the hour. This editorial accentuates different novel strategies with emphasis on gene therapy and immunotherapy for the management of CRC.

Key words: Colorectal cancer; Immunotherapy; Gene therapy; Signaling; Targeted therapy

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INTRODUCTION

Colorectal cancer (CRC) is the third most predominant cancer amongst the world. In 2017, 97220 and 43030 new patients of colon and rectum cancers were reported in United States, respectively. CRC is manifested by the development of adenomatous polyps and malignant cells in the colon. These abnormal cells producing tumors are characterized by uncontrolled replication and the property of metastasis. The early detection, diagnosis, and the utilization of efficient and safe delivery systems would tremendously enhance the efficacy of therapy. The novel targeting approaches (Figure 1), of raising concern as manifested by cancer drugs in the past years, block transduction pathways leading to the cell death through apoptosis and triggering of the immune system, or deliver anticancer drugs to cancer cells, reducing the side effects. The major pathways which could be targeted for CRC therapy are, Sonic Hedgehog (SHH), Wnt/β-catenin, transforming growth factor-β (TGF-β)/SMAD, EGFR and Notch pathways. Novel targeting approaches and signaling pathways of colorectal cancer: An insight. World J Gastroenterol 2018; 24(39): 4428-4435 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i39/4428.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i39.4428

Core tip: In spite of the advancements in the diagnosis and the treatment approaches for colorectal cancer (CRC), its survival rate is quite low. Therefore, there arises an urge to develop novel targeting strategies for its effective treatment. A meticulous apprehension of the signaling cascade is necessitated for better outcomes. In a nutshell, this editorial highlights various novel targeting approaches like gene therapy and immunotherapy which could usher better targeting of CRC.

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Frizzled (Fz) receptors and low-density lipoprotein receptor-related protein 5 or 6 (LRP5 or LRP6) are the targets of the Wnt family of proteins. The primary element of the Wnt/β-catenin signaling pathway is the β-catenin destruction complex; which is comprised of a tumor suppressor protein encoded by the antigen-presenting cells (APC) gene, Axin, CK1, and GSK3β. When the receptor binding does not occur, this complex undergoes binding with the β-catenin protein (encoded by CTNNB1 gene), which then undergoes degradation through an ubiquitin-proteasome pathway. In contrary, binding of the receptor by Wnt ligands causes the deactivation of the β-catenin destruction complex and accumulation of β-catenin. It is then translocated to the nucleus for complex formation with T-cell factor/lymphoid enhancer factor, a transcription factor, causing the transcriptional actuation of the target genes. In majority of colon cancers (sporadic) mutation of both alleles of APC (a tumor suppressor gene) occurs which leads to stabilization of β-catenin and stimulation of WNT pathway genes, like TCF, which are needed for the maintenance of colon crypt. In few colon cancers identification of point mutation in β-catenin bearing wild-type alleles of APC has been done. Aquaporin5 (AQP5), a water protein channel, has an oncogenic activity in many types of malignant cancers like CRC. The effect of AQP5 silencing on 5-fluorouracil (5-FU) sensitivity was inquired in cancer cells. It was observed that the Wnt/β-catenin pathway mediated the 5-FU chemosensitivity. AQP5 silencing suppressed the Wnt pathway. While, overexpression of the β-catenin (S33Y) mutant (which shows resistance to degradation) reversed the apoptosis process triggered by AQP5 silencing. Berberine, which is an alkaloid derived from plants and its synthetic 13-arylalkyl derivatives have been accounted to possess antitumor potential; they were investigated for their involvement in Wnt/β-catenin signaling cascade. The cellular levels of active β-catenin were found to decrease accompanied by a rise in the expression of E-cadherin. The berberine derivatives depicted a 100-times reduced EC50 values in comparison to berberine for Wnt-repression. Esculetin, (6, 7-dihydroxycoumarin) potentially inhibits the Wnt/β-catenin signaling pathway. It interrupted the β-catenin-Tcf complex formation by binding with the Lys312, Gly307, Lys345, and Asn387 residues of β-catenin in tumor cells. Besides, esculetin efficaciously reduced the viability and suppressed the anchorage-independent proliferation of cancer cells. Novel Wnt signaling inhibitors, isopropyl 9-ethyl-1- (naphththalen-1-yl)-9H-pyrido (3, 4-b) indole-3-carboxylate (Z86) have been recognized. Z86 suppressed the Wnt signaling functions and genes expression in mammalian cells. It suppressed the GSK3β (Ser9) phosphorylation, causing its overactivity and elevating the phosphorylation and β-catenin degradation.

TGF-β and BMP signaling pathways are often impaired in CRC. Ligand-induced oligomerization of the TGFBR1

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TGF-β and BMP signaling pathways are often impaired in CRC. Ligand-induced oligomerization of the TGFBR1
serine/threonine receptor kinases leads to the initiation of the signal cascade succeeded by the phosphorylation of Smad1, Smad2 and Smad3 (signaling molecules). This leads to their association with Smad4 (signaling transducer) and translocation to the nucleus. Triggered Smads modulate various biological effects by binding to transcription factors and leading to the modulation of transcription. Juvenile polyposis is observed in colon cancer due to mutated Smad4 or BMPRI. In most of sporadic colon cancers, the phosphorylation of Smad1, Smad5 and Smad8 does not occur\(^\text{[10]}\). Genistein (obtained from soybean) is an isoflavone possessing an anticancer potential. A dose-dependent rise in TGF-beta1 mRNA expression was found in MC-26 cells in mouse. It stimulated the generation of Smad-DNA complexes and phosphorylated Smad2 and Smad3, depicting enhanced TGF-beta1 signaling\(^\text{[11]}\).

The binding of epidermal growth factor and TGF to the EGFR, leads to the stimulation of homodimerization/heterodimerization of the receptor and phosphorylation of specific tyrosine residues (P). This in turn stimulates the downstream RAS/RAF/mitogen-activated protein
kinase (MAPK) and phosphoinositide 3'-kinase (PI3K) signaling pathways and expression of genes responsible for cell proliferation, angiogenesis and metastasis. KRAS2 and BRAF mutations have been seen in colon cancer. Mutations in PIK3CA which is the p110α catalytic subunit of PI3K have also been observed in few cases of colon cancers\(^{[12]}\). Everolimus (an inhibitor of mTOR) in combination with nilotinib (a platelet-derived growth factor receptors tyrosine kinase inhibitor) suppressed the growth and liver metastasis of colon cancer. The stromal reaction and cancer cell proliferation was reduced and apoptosis was stimulated in tumor cells\(^{[13]}\).

The Notch signaling pathway is involved in the growth of intestinal epithelium. Notch ligands i.e., Delta-like (DLL) bind to their transmembrane receptors (Notch 1-4) and induce the proteolytic breakdown of the receptors by the enzymes α-secretase and γ-secretase to release the intracellular domain of the Notch receptor. The cleaved Notch receptors (NICD) are then transferred into the nucleus which forms complexes with RBP-Jk (CSL or CBF-1) and lead to the stimulation of Notch-target gene Transforming growth factor-β. An overexpression of ligands namely Jagged1, Jagged2, DLL1, DLL3, DLL4, Notch receptors 1-4 and genes like hairy-enhancer-of-split (Hes-1), Deltaex and Notch intracellular domain (NICS) has been observed in colorectal cancer cells\(^{[14]}\). Withaferin-A is a natural compound (source Withania somnifera), which curbs Notch-1 signaling and downregulates various pathways like Akt/NF-kappa B/Bcl-2, in HCT-116, SW-480, and SW-620 cell lines. Besides, Withaferin-A downregulated the expression of mammalian target of rapamycin (mTOR) signaling components, pS6K and p4E-BP1, and stimulated c-Jun-NH (2)-kinase-mediated apoptosis in tumor cells\(^{[15]}\).

### TARGETED THERAPY

Nanotechnology is a rising arena in drug delivery which furnishes many advantages over the conventional system. Colon-specific novel delivery systems would allow for the local delivery of a high concentration of drugs in the colon to improve pharmacotherapy and reduce its potential systemic toxicity and side effects. Recently, theranostic nanocarriers are introduced to simultaneously monitor and treat the disease using a single delivery system\(^{[16]}\). Colon targeted nanocarriers have been described in brief in the Table 1\(^{[17-26]}\).

### GENE THERAPY

It involves introduction of genetic components for treating various diseases including cancer. The genetic component may be the nucleic acid i.e., DNA or RNA which may help to replace or correct the malfunction due to defective genes. Gene therapy can also be utilized to actuate an immune response or itself used as a therapeutic agent.

Progression of colorectal cancer is mediated by mutation and aberration of genes. Modification and correction of these defective genes and prevention of those overexpressed genes can have the capability to prevent CRC. The alteration of multiple genes is involved in the development of colon carcinogenesis. Point mutation, formation of oncogenes, de-regulation or deletion of proto-oncogenes and lack of function of suppressor-oncogenes may lead to cancer. Till November 2017, near about 2600 clinical trials had been conducted in 38 countries and more than 50% are in phase 1 clinical trial\(^{[27]}\). While 1309 gene therapy based trials which were performed across the

### Table 1 Nanotechnology based drug delivery systems for colorectal cancer targeting

| System                  | Chemotherapeutic agent | Significance                                                                 | Ref.  |
|-------------------------|------------------------|-------------------------------------------------------------------------------|-------|
| Nanoparticles           | Resveratrol (RSV)      | Sustained release of RSV (over 72 h), and drug solubility enhancement         | [17]  |
| Micellar delivery system| Docetaxel              | Enhanced the efficacy of hydrophobic chemotherapy and reduced systemic toxicity| [18]  |
| Self-nanoemulsifying drug delivery systems (SNEDDS) | Sunitinib malate | Enhancement of *in vitro* dissolution rate and anticancer potential of drugs possessing low water solubility such as sunitinib malate | [19]  |
| Small molecule-based theranostic system, Gal-Dex | Doxorubicin | Drug localization and site of action can be monitored | [20]  |
| Polymeric micelles      | Tanshinone IIA (TAN)   | Improved efficacy of anticancer drugs and promoted the growth of beneficial commensal flora in the gut | [21]  |
| Pressure-sensitive nanogels | 5-Fluorouracil (5-FU) | Higher 5-FU intracellular accumulation and a significant cell death extension by apoptosis | [22]  |
| Microspheres            | Atorvastatin and celecoxib | Synergistic effect on colon cancer prevention and inhibition | [23]  |
| Microbeads              | Doxorubicin            | Exhibited reduction-responsive character, release the DOX in reducing environments due to cleavage of the disulfide linkers | [24]  |
| Carboxymethyl dextran (CMD) chitosan nanoparticles | Small interfering RNA | Significant changes of Epithelial mesenchymal transition genes and apoptosis | [25]  |
| Liposomes               | Apatinib               | cRGD-modified liposomes displayed greater apoptosis                          | [26]  |
IMMUNOTHERAPY

Tumor immunotherapy has seized researchers in this scenario as it depicts remarkable clinical potential in CRC. Presently, there are various immunotherapies which are being subjected to clinical trials in human CRC. Various immunotherapy approaches employed in CRC are monoclonal antibody (mAb) therapy, immune checkpoint inhibitors therapy, cancer vaccines, adoptive cell therapy, complement inhibition and cytokine treatment. Majority of them are in phase I and II clinical trials and some of these trials showed promising results. So far, more than 24 immunotherapy-based clinical trials for human CRC have been completed and more than 40 clinical trials are recruiting or about to recruit patients[33]. Table 2[34-40] depicts various clinical studies of CRC.

**Monoclonal antibody therapy**

In this therapy, humanized antibodies like Cetuximab and Panitumumab which selectively recognize the epidermal growth factor receptor (EGFR) are employed for the treatment of metastatic CRC. There are some mAbs presently in various phases of clinical trials for CRC such as adocatumumab against EpCAM, labetuzumab against carcinoembryonic antigen (CEA), and pertumomab against Mucins[41].

**Immune checkpoint inhibitors therapy**

T cell activation is down-regulated by CTLA-4 which is an immune checkpoint moiety by binding to CD80/CD86 entities on antigen-presenting cells (APC). T cell function is negatively regulated by programmed death receptor ligand 1/2 (PD-L1/L2) by binding to PD-1 receptor present on T cells usually stimulated by their various ligands which are expressed on either tumor cells (e.g., PDL1/2→PD-1) or APCs (e.g., CD80/86→CTL-A4; PD-L1/L2→PD-1), activated CTLA-4 and PD-1 immune checkpoint signaling pathways efficiently inhibit the tumor-reactive T cell activation and consequent tumor detection[42]. A phase II clinical trial of individual drug Nivolumab and also a combination of dual drugs like Nivolumab plus Ipilimumab is in undergoing process for CRC (ClinicalTrials.gov Identifier: NCT02060188).

**Cancer vaccines**

They have been designed to induce antigen specific T-cell or B-cell activity against cancer by rendering antigens to APC like dendritic cells (DCs). Besides, vaccines likewise include constituents proposed to activate DCs pulsed with antigens and aim them to move to a local lymph node.eg DC vaccine and OncoVAX.

**DC vaccine**: Because majority of CRCs express carcinoembryonic antigen (CEA) which is a tumor-associated antigen DCs, can be pulsed with CEA mRNA or CEA peptides. Most of the CRC patients who were administered with DC vaccine evoked CEA-specific T cell immune activities.

**Oncovax**: It has been developed to use patients’ own cancer cells with an immune-stimulating adjuvant to evoke antitumor immune activities to evade the relapse of colon cancer after surgery. A combination of specific immunotherapy with surgery depicts a remarkable improvement in the survival of the patients[43].

**Adaptive T cell therapy**

This therapy possesses the potential to raise antitumor immunity and increase vaccine efficacy. Recent researches have riveted on endowing effector T cells with desired antigen receptors, like chimeric antigen receptor T cells. An ex vivo expanded human Vδ1 γδ T cells displayed a remarkable therapeutic activity in

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Table 2  Overview of clinical trials of colorectal cancer

| Therapy                                                                 | Agent                                             | Clinical status | Ref.   |
|------------------------------------------------------------------------|----------------------------------------------------|-----------------|--------|
| Five peptides combination with oxaliplatin-based chemotherapy          | Oxaliplatin                                        | Phase II        | [34]   |
| Panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) vs FOLFOX4 alone | Fluorouracil, Oxaliplatin                          | Phase III trial | [35]   |
| Checkpoint inhibitors                                                    | Nivolumab and pembrolizumab                       | Phase 2 study   | [36]   |
| Combination vaccine treatment of five therapeutic epitope-peptides      | Fluorouracil, irinotecan or oxaliplatin            | Phase I         | [37]   |
| Autologous dendritic cell based adoptive immunity                       | -                                                  | Phase I-II      | [38]   |
| Autologous antigen-activated dendritic cells in the treatment of CRC    | -                                                  | Phase I-II      | [39]   |
| Adjuvant chemotherapy (FOLFOX)                                           | 5-fluorouracil (FU)/leucovorin (LV)               | Phase III       | [40]   |
Complement inhibition
Complement is a key part of the immune system and its stimulation has been taken as an essential component of the immune surveillance response against CRC. Complement comprises of more than 30 proteins and fragments, is part of the innate and adaptive immune system. Various protein inhibitors of complement such as cobra venom factor, humanized cobra venom factor, and recombinant *staphylococcus aureus* super antigen-like protein 7 have been assessed in murine colon cancer models. Complement depletion presents an efficient type of immunotherapy in CRC by its capability to vitiate tumor progression by raising the host’s immune responses to cancer and reducing the immunosuppressive effect generated by the tumor microenvironment and finally could be employed as a constituent of combination immunotherapy.[46]

Cytokine therapy
Cytokines are considered as essential aspects of tumour immunology, particularly for CRC, in which the tumor growth is determined by the inflammatory process and immunogenic responses. Cytokines like tumour necrosis factor and interleukin-6 are considered as important factors in CRC, triggering the stimulation of the central oncogenic factors nuclear factor-κB and inducer of transcription 3 (STAT3), respectively, in the intestinal cells to enhance the proliferation and the development of apoptosis resistance[46] (Figure 3).

CONCLUSION
Increasing evidences show that several signaling pathways play an essential role in the development and progression of CRC. Targeting these signaling cascades using nanocarriers might be advantageous for the treatment of CRC. The identification of various genes and other biomarkers improved the conventional therapy and target the specific tumor cells. The gene therapy and various immunotherapy including cytokine therapy, cancer vaccine, adoptive cell therapy, monoclonal antibody etc. have been recently introduced which may unravel new ways for the treatment of CRC and provide its efficient management in comparison to the conventional therapy.

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