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=== REVIEW ===

Strategies to improve the efficacy of curcumin in colorectal cancer treatment

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SUMMARY. Colorectal cancer is a severe type of disease, in which surgical therapy complemented by radio- or chemotherapy, is hindered by the chemoresistance or secondary effects. Due to the complex and dynamic interactions in tumor microenvironment, there is constant need in designing new anti-cancer strategies that simultaneously target directly cancer cells development and indirectly the pro-tumor processes mediated by the crosstalk of cells in tumor milieu. Curcumin, is a natural, biological safe polyphenol, with anti-tumor, pro-apoptotic and immunomodulatory actions in a wide spectrum of neoplasia including colorectal cancer. Specifically, its ability to orchestrate the processes associated with tumorigenesis such as cancer cell proliferation, metabolism, angiogenesis, inflammation, oxidative stress and immunosuppression, has been largely documented, but insufficiently exploited. However its use in preclinical and clinical studies is hindered due to low solubility in aqueous environments, poor absorption, instability and high rate of degradation. In this article we review the existing data on the anti-tumor actions of curcumin in colorectal cancer and potential strategies aiming at enhancing its efficacy in the treatment of this disease. Due to its ability to both prevent and treat colorectal cancer, by modulating multiple targets, active delivery of curcumin or curcumin analogues combined with other chemotherapeutic agents, is a promising therapeutic approach for this type of cancer, with minimal toxicity to healthy tissues.

Keywords: CRC, curcumin, cytotoxic actions, nanoformulations.

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Introduction

Colorectal cancer (CRC) is a severe chronic disease of the digestive tract affecting more than 1.2 billion people worldwide. The mechanisms responsible for CRC pathogenesis, specifically the neoplastic transformation of normal cells, proliferation, new blood vessel formation, invasion and metastasis have been attributed to genetic and epigenetic factors, and equally to oxidative stress, inflammatory and metabolic processes (Haggar and Boushey, 2009). The therapeutic approach for this type of cancer consists in tumor surgical resection complemented by radiotherapy and/or systemic administration of cytotoxic agents (5-fluorouracil, oxaliplatin, capecitabine), which, unfortunately, due to the lack of specificity for cancer cells, have significantly secondary effects. Additionally, some patients develop resistance to chemotherapeutic agents among which 5-fluorouracil (5-FU) (Chibaudel *et al.*, 2012). Co-administration of chemotherapeutic agents with naturally occurring drugs, which are pharmacologically safe, may overcome conventional chemotherapy side effects (Fantini *et al.*, 2015). In this sense, efficient adjuvant strategies for chemotherapy have been ascribed for flavonoids, polyphenols, stilbenes and other natural compounds (Aggarwal *et al.*, 2013). Among these, **curcumin**, an active polyphenol isolated from *Curcuma longa* possesses anti-tumor and immunomodulatory actions in gastric, cervical, melanoma, genitourinary, breast, esophagus, lung, neurological, hematological and intestinal cancers (Tuorkey, 2014). Curcumin pharmacological activities translate in inhibition of processes entertaining cancer cells development such as cell proliferation, epigenetic or metabolic processes, inflammation, angiogenesis, oxidative stress, invasion and metastasis.

In this article we review the anti-tumor actions of curcumin in CRC and strategies aiming at improving its efficacy in the treatment of this disease.

Thus, in the first part of this work we describe the main effects of curcumin on modulating tumor associated processes such as cell growth, apoptosis, metabolic and epigenetic alterations, inflammation, angiogenesis, and oxidative stress, followed up by a presentation of therapeutic strategies exploiting these effects.

1. Anti-tumor effects of curcumin that modulate tumor growth- associated processes

Modulation of cancer cell growth and apoptosis

Through its remarkable ability to modulate NF-kB expression and activation curcumin attenuates CRC cells (HCT116, HT29, Caco-2) growth by interfering with cell cycle regulatory proteins, arresting the cells in the G1, S/G2 or G2/M phase (Aggarwal *et al.*, 2003). Subsequentially, curcumin induces apoptosis by inhibiting

the expression of anti-apoptotic factors Bcl2, Bcl-xl, stimulating the expression of pro-apoptotic proteins: Bax, Bal, Bok, p21, p27 and (but not necessary) tumor suppressor p53 (Moos *et al.*, 2004; Shehzad *et al.*, 2013), activating caspase 3 and finally inducing the release of cytochrome c from mitochondria (Kunnumakkara *et al.*, 2008). Additionally, modulation of signaling pathways controlled by EGFR (Chen *et al.*, 2006), COX2 (Goel *et al.*, 2001), MAPKs, AMPKs and Wnt/β catenin (Collett and Campbell, 2004; Jaiswal *et al.*, 2002) or interfering with the ubiquitin-mediated degradation of proteins in the proteasome machinery, are effects described for curcumin induced-apoptosis (Hasima and Aggarwal, 2014).

Modulation of epigenetic events

Targeting epigenetic events in cancer cells is nowadays a strategy to prevent aberrant DNA methylation, histone acetylation/deacetylation, or miRNA expression (Lao and Grady, 2011; Vaiopoulos *et al.*, 2014). Curcumin is a recognized inhibitor of these epigenetic alterations exerting its anti-cancer effect at least in part through epigenetic modulation of global DNA hypomethylation or local DNA-hypermethylation in human HCT116 and HT29 CRC cells (Guo *et al.*, 2015b; Link *et al.*, 2013). Aberrant acetylation/deacetylation affecting histones or non-histones proteins, occur post-translationally and have been associated with CRC (Sadoul *et al.*, 2008). Curcumin has been found to be a potent inhibitor of the activity of both histone acetyltransferases and histone deacetylases (Reuter *et al.*, 2011). miRNAs are short, non-coding RNAs regulating post-transcriptionally the gene expression. Their altered expression has been associated with cell proliferation, growth, angiogenesis, migration and apoptosis of cancer cells. They have both oncogenic or tumor suppressor activities, which can be regulated by various agents, offering great anti-tumor therapeutic perspectives. While, in some studies, curcumin or its analogues have been demonstrated to downregulate oncogenic miRNA21 involved in migration, invasion and proliferation of HCT116 CRC cells, in other studies curcumin upregulated the tumor suppressive miRNA34a, miRNA27a, thus inhibiting cancer growth *in vitro* and *in vivo* (Gandhy *et al.*, 2012; Toden *et al.*, 2015a; Toden *et al.*, 2015b).

Modulation of inflammatory pathways and angiogenesis

It has long been recognized that targeting tumor-associated inflammation and angiogenesis, with different compounds (statins, glucocorticoids, non-steroidal anti-inflammatory drugs etc.) is both an attractive and efficient therapeutic anti-cancer strategy (Banciu, 2007; Rayburn *et al.*, 2009). In CRC tumor inflammation is driven mainly by the overexpression of the ubiquitous transcription factor NF-kB

(Voboril and Weberova-Voborilova, 2006). Curcumin inhibits the production of soluble mediators produced by tumor cells or the tumor-associated cells, entertaining a pro-inflammatory milieu (Casey *et al.*, 2015), by inhibiting, *in vitro* and *in vivo*, the activation by phosphorylation of NF-kB and its downstream inflammatory regulated effectors: COX-2, TNF-α, IL-6, PGE_2, MMP3, MMP9, ROS, iNOS and most importantly VEGF (Aggarwal *et al.*, 2006). VEGF, the major pro-angiogenic factor, is synthetized and activated by hypoxia, inflammation, oxidative stress, and other growth factors (bFGF, EGF, TGF-B, PDGF-BB). Curcumin has been shown to inhibit the activation of HIF-1α, which is constitutively express in hypoxic area of solid tumors, therefore inhibiting its main targets- VEGF or bFGF, MMP-1,2,3,9 and TIMP (Yadav and Aggarwal, 2011). In melanoma or colon tumors, macrophages have been shown to dominate the inflammatory infiltrate, manifesting a dual phenotype, depending on the localization and stage (Sica and Mantovani, 2012). They are equally responsible for sustaining tumor development by producing IL-1, IL-6, IL-10, TNF-α, IL-21, VEGF, TGF-b, MMPs, ROS, NO, or impairing it by phagocytosis of cancer cells or production of anti-inflammatory/anti-angiogenic factors (Erreni *et al.*, 2011). Curcumin has the ability to reeducate tumor associated macrophages as shown in animal models for breast cancer, activating their intrinsic anti-tumor functions (Shiri *et al.*, 2015; Zhang *et al.*, 2013). This offers great therapeutic perspectives for CRC treatment.

Modulation of invasion and metastasis

Cancer cells migration process at secondary sites is tightly controlled by growth factors, cytokines and cell adhesion molecules, as well as intracellular signaling systems (Chambers *et al.*, 2002). Among these up- regulation of NF-kB expression and deregulation of the Wnt/β-catenin signaling pathway, which is a major regulator of the cell proliferation, motility and migration, has been suggested to be a major cause of malignant dissemination (Chen *et al.*, 2013). Similarly, loss of E-cadherin and enhanced activity of matrix metalloproteinases contribute to tumor cells ability to invade and metastasize. Curcumin was shown to affect both Wnt signalling and cell-cell adhesion pathways (Jaiswal et al., 2002; Narayan, 2004) in human CRC cell lines (HCT-116, HT-29, HCT-15, HCC-2998, Colo205) or in animal models, inhibiting NF-kB, PKC, RhoA, MMP-2, MMP-9, COX2 gene expressions and enhancing E-cadherin expression, thereby preventing cancer cell invasion and metastatic potential (Shen *et al.*, 2014). Recently, it has been shown, that curcumin exerts anti-metastatic effects by modulating the TGF- β-mediated crosstalk between human HCT116 CRC cells and human fibroblasts (MRC-5) in co-culture. TGF-β is a major metastatic promoter of cancer cells (Buhrmann *et al.*, 2014).

Modulation of reactive oxygen species production

In cancer cells, low level of ROS foster the survival and maintenance of cellular viability and phenotype, whereas aberrant ROS production induces a cellular redox imbalance, which causes macromolecular damage and finally, cell death (Martindale and Holbrook, 2002). Curcumin functions, at low concentrations as an antioxidant (upregulating the antioxidants levels) whereas at higher concentrations curcumin manifests prooxidant cytotoxic activity (Das and Vinayak, 2014). This is due to curcumin ability to interfere with the expression and activation of different transcription factors (NF-kB, AP-1, Nrf-2), their downstream effectors (COX-2, MMPs, iNOS, VEGF, PPAR-γ) or signaling pathways (ERK, PI3K/Akt, JNK) (Lin, 2007; Surh, 2003). ROS-mediated cytotoxicity of curcumin has been demonstrated in human CRC cell lines (Colo205, HCT116, HCT115, HT29) or animal models (Su *et al.*, 2006).

Modulation of tumor energy metabolism

A hallmark of neoplastic transformed cells, is the deregulated energy metabolism. Cancer cells consume much higher level of glucose, fatty acids and glutamine to ensure their anabolic growth (DeBerardinis *et al.*, 2008). Glycolysis, the main energy furnisher in cancer cells, results also in generation of high levels of lactate and H^+ which in turn acidifies the tumor microenvironment promoting tumor invasion, as well as precursors for the synthesis of nucleotides or fatty acid synthesis (Zhao *et al.*, 2013). In addition to glucose, cancer cells rely also on glutaminolysis to support their growth, as glutamine is an important aminoacid fueling the TCA cycle (Phan *et al.*, 2014). Almost all of the glycolysis and TCA cycle participants, have been linked with cancer cell growth, invasion, metastasis (Kim and Dang, 2005). In several cancer models, the glucose transporters (Glut1, Glut3, Glut4) (Macheda *et al.*, 2005) and/or glycolytic enzymes (HKII, PFK, GAPDH, PK, LDH) (Zhang and Yang, 2013) are upregulated by cooperation of c-MYC and HIF-1α. MYC-enhanced glutamine catabolism is also observed (Kim *et al.*, 2007). Inhibiting key metabolic enzymes in CRC cells, would offer great therapeutic perspectives, as a recently published study from Wang *et al.*, 2015, describes pro-apoptotic action of curcumin on human HCCT116 and HT29 CRC cells, mediated by direct inhibition of the rate limiting glycolytic enzyme Hexokinase II in an Akt-dependent manner (Wang *et al.*, 2015). Moreover, administration 5-FU and dicholoracetate has already been shown to inhibit pyruvate dehydrogenase kinase *in vitro*, in human CRC cell lines LS174T, LoVo, SW620, and HT29 (Tong *et al.*, 2011). It is though tempting to speculate, that a powerful anti-cancer effect would result from the co-treatment of cancer cells with curcumin and 5-FU, in an attempt to blunt their energy metabolism. However, there should be great concern regarding the specificity of such treatments, as glycolysis occurs in both neoplastic and healthy cells.

2. Strategies aiming at improving the efficacy of curcumin in CRC treatment

Curcumin administration in combination with cytotoxic agents

It was demonstrated that the administration of therapeutic agents in combination usually engenders a greater anti-tumor effect, over monotherapy. Chemotherapeutics in CRC include oxaliplatin, irinotecan, capecitabine and 5-FU. Experimental studies show that curcumin is able to synergize with some of these agents, in the anti-tumor actions (Patel and Majumdar, 2009). Particularly, enhanced apoptotic effects were observed for 5-FU who remains the main chemotherapeutic agent for the treatment of both colorectal and breast cancer. Its anti-tumor effect in mammalian cells results from its ability to block the activity of thymidylate synthase, an enzyme involved in DNA replication and repair, leading to inhibition of cellular growth and apoptosis (Longley *et al.*, 2003). 5-FU clinical efficiency is limited by its low specificity for the target tumor tissue, the low biodisponibility, and especially the accelerated degradation by the liver. Additionally, the fact that tumor cells often develop resistance to this chemotherapeutic agent, by activating other salvaging signaling pathways, impedes the successful treatment of CRC in suffering patients (Malet-Martino and Martino, 2002). Curcumin or its analogues administrated as an adjunct to the chemotherapeutic drug 5-FU, offers a promising strategy for the treatment of CRC. *In vitro* studies on human CRC cell lines HCT116 envisage the ability of curcumin co-administrated to 5-FU, to reduce the proliferation and viability of cancer cells and to induce apoptosis by blocking the expression and activity of the constitutively activated NF-kB (Shakibaei *et al.*, 2013). When combined with oxaliplatin, curcumin is able to inhibit colon carcinoma *in vivo*, in nude mice xenografted with human CRC cells (LoVO) in an apoptotic manner (Guo *et al.*, 2015a). Multiple targeting of human CRC cells HCT116 or HT29, with 5-FU, oxaliplatin and curcumin, resulted in higher cytotoxic effects than each of the individual or dual treatments. Altered EGF-R, IGF-1R, Akt signaling pathway or COX-2 expression were associated with this cytotoxic action (James *et al.*, 2015; Shakibaei *et al.*, 2015). Additionally, inclusion of curcumin in conventional therapeutic regimens is an effective strategy to reverse the chemoresistance of cancer cells (Goel and Aggarwal, 2010). Curcumin reverses the multidrug resistance of CRC cells to vincristine, cisplatin, and hydroxycamptothecin *in vitro* and *in vivo* (Lu *et al.*, 2013) and enhances the chemosensitivity to 5-FU in human CRC cells (HCT116 or HT29) (Shakibaei *et al.*, 2014; Shakibaei *et al*., 2015; Toden *et al*., 2015b). Moreover, when applied on CRC cells and MRC-5 fibroblasts co-culture in a monolayer or high density tumor microenvironment model *in vitro*, curcumin and 5-FU were able to suppress cell synergism in tumor microenvironment and sensitize the cells to 5-FU (Buhrmann *et al*., 2014).

Administration of synthetic curcumin analogues

Another approach to overcome pharmacological problems for curcumin and enhance its efficacy is the design of new compounds which are superior to curcumin in the anti-tumor actions in various *in vitro* and *in vivo* models for colorectal tumorigenesis,

while retaining their non-toxicity to healthy tissues. Dimethoxy-curcumin, diphenyl-difluoroketone-EF24, hexahydroxycurcumin, difluorinated-curcumin are some examples of analogues of curcumin, chemically modified in relation to the parent compound (by methylation, reduction, condensation etc), found to affect cancer cells development with higher potency than curcumin. In HCT116 CRC cells dimethoxy-curcumin inhibited proliferation and induced apoptosis (Tamvakopoulos *et al.*, 2007). Diphenyl-difluoroketone tested on HCT116 and HT29 CRC cells, manifested anti-tumor actions by arresting the cells in G2/M phase of the cell cycle, reducing VEGF and COX2 expression, and inducing caspases- mediated apoptosis (*in vitro* and *in vivo*) (Subramaniam *et al.*, 2008). Another derivative of curcumin-hexahydroxycurcumin combined with 5-FU exhibited potent anti-tumor activity on HT29 human CRC cells by inhibiting COX2 mRNA and protein expression (Srimuangwong *et al.*, 2012). Moreover, difluorinated curcumin (CDF), was shown to inhibit the growth of CRC cells resistant to 5-FU and oxaliplatin, due to reduced expression of miR-21, therefore sensitizing the cells to these chemotherapeutics (Roy *et al.*, 2013).

Encapsulation of curcumin in nanoparticles

Although curcumin has theoretically, a huge therapeutic potential, its low solubility in aqueous environments, poor absorption, the high degree of instability and hepatic and intestinal rate of degradation limit its uses in preclinical and clinical studies (Shehzad *et al.*, 2010). To overcome these issues and to substantially improve curcumins biological activity different nanoformulations have been developed with aim of passively or actively targeting cancer cells as presented in detail in Table 1. For a detailed overview of these formulation please consult (Yallapu *et al.*, 2013). For example polymeric nanoparticles, liposomes, cyclodextrines, etc, have been ascribed to efficiently incorporate curcumin, stabilize the compound, enhance its cellular uptake, and cytotoxicity (Yallapu *et al*., 2013).

Moreover, to actively target the tumor cells with curcumin, functionalized bioconjugates have been also developed. One such hibrid formulation contains a hydrophobic core (poly(D,L-lactide-co-glycolide)(PLGA), a lipid based monolayer surrounding the PLGA and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-carboxy (polyethylene glycol) 2000 (DSPE-PEG_{2000}-COOH)-shell. This shell enhances the half-time of curcumin and binds a small RNA fragment (Aptamer) directed agains adhesion molecules overexpressed on CRC cells. Enhanced cellular uptake and cytotoxicity for this nanoformulation has been reported on HT29 human CRC cells (Li *et al*., 2014a). In colorectal cancer, administration of 5-FU as a free drug has a poor therapeutic effect due to the lack of the tumor site specificity, rapid metabolization/ degradation and associated side effects. Subsequently, many nano-formulations of the drug were developed with the aim to shorten the main drawbacks we mentioned as shown in Table 1 and described in detail elsewhere (Arias, 2008).

Table 1.

Curcumin and 5-FU nanoformulations used in CRC treatment

Nano-formulations for delivering curcumin	*Pre-clinical study*	*References*
Liposomes	*In vitro*, *in vivo*	(Lin *et al.*, 2012; Rahman *et al.*, 2012)
Pluronic/Polycaprolactone micelles	*In vitro*	(Raveendran *et al.*, 2013)
Polymeric micelles in thermosensitive hydrogel system	*In vitro*, *in vivo*	(Zhang *et al.*, 2015)
Polymeric nanoparticles	*In vitro*, *in vivo*	(Tan *et al.*, 2014)
Albumin nanoparticles	*In vitro*	(Kim *et al.*, 2011)
Thiolated chitosan nanoparticles	*In vitro*, *in vivo*	(Anitha *et al.*, 2014)
Cyclodextrin complexes	*In vitro*	(Yadav *et al.*, 2010)
Glycerol monooleate and pluronic F-127 based nanoparticles	*In vitro*	(Mohanty and Sahoo, 2010)
Silica nanoparticles	*In vitro*	(Singh *et al.*, 2015)
Eudragit S100 nanoparticles	*In vitro*	(Prajakta *et al.*, 2009)
Chitosan and gum arabic nanoparticles	*In vitro*	(Udompornmongkol and Chiang, 2015)
PLGA-lecithin-PEG–Apt-nanoparticles		(Li *et al.*, 2014a)

Nano-formulations for delivering 5-FU	*Pre-clinical study*	*References*
Enteric-coated chitosan polymeric nanoparticles	*In vitro* drug release studies	(Tummala *et al.*, 2015)
Polymeric hydrogels	*In vitro*	(Mishra *et al.*, 2014)
Poly(ε-caprolactone) nanoparticles	*In vitro*	(Ortiz *et al.*, 2012)
Solid lipid nanoparticles	*In vitro*	(Yassin *et al.*, 2010)
Thiolated Chitosan nanoparticles	*In vitro*, *in vivo*	(Anitha et al., 2014)
Hyaluronic acid coupled chitosan nanoparticles	*In vitro*	(Jain and Jain, 2008)
Chitosan nanoparticles	*In vitro*	(Li *et al.*, 2014b)
Magnetoliposomes	*In vitro*	(Clares *et al.*, 2013)
Layered double hydroxide nanoparticles	*In vitro*	(Chen *et al.*, 2014)

Conclusions

Curcumin is a promising natural compound able to target multiple processes associated with CRC. To remarkably improve its efficacy, co-administration with chemotherapeutic agents or the use of non-toxic analogues of curcumin, which, as described, seem to be superior in action to curcumin, might be a reasonable approach for CRC treatment. However, an active targeting strategy, by using functionalized nanoformulations, remains until now, the best alternative, to take advantage of curcumin cytotoxic effects on cancer cells. To demonstrate the clinical potential of such formulation, remains to be properly assessed.

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