Journal of Cancer Science & Therapy
ISSN: 1948-5956

Reed et al., J Cancer Sci Ther 2019, 11:7

Review Article Open Access

An Evaluation of Innate Immune Anomalies in Colorectal Tumorigenesis and Precision Medicine

Dominique Z Reed[1,2,3], Nicole Krupp[4], Suman Suman[1], Susan Yeyeodu[5,6], Kevin S Kimbro[5] and LaCreis R Kidd[1,2]*

[1]*Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY, USA*
[2]*James Graham Brown Cancer Center, Louisville, KY, USA*
[3]*Department of Pharmaceutical Sciences and Skaggs School of Pharmacy, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA*
[4]*Vanderbilt University Medical Center, Nashville, TN, USA*
[5]*North Carolina Central University, Julius L. Chambers Biomedical and Biotechnology Research Institute, Durham, NC, USA*
[6]*Charles River Discovery Services in Morrisville, NC, USA*

Abstract

Colorectal cancer is the fourth leading cause of cancer-related deaths. Once the disease spreads and becomes less responsive to conventional treatments, the 5-year survival rates drops to 13%. Thus, new therapeutic targets will help to exert anti-tumor effects or improve the effective treatment of aggressive colorectal cancer. Ideal clinical management tools include Toll-like receptor-related markers (e.g., TLR3, TLR5, TLR7, TLR8, TLR9, MyD88) due to their capacity to alter numerous cancer-related pathways, including innate/adaptive immune/inflammation signaling, cell death, cell proliferation, DNA repair, cell migration, angiogenesis, and metastasis. This review sheds light on the immune boosting and anti-carcinogenic effects of TLR agonist/antagonist alone or combined with conventional therapy (radiation, chemotherapy, antibiotics) against colorectal cancer based on pre-clinical and clinical studies. Additional research is needed on the therapeutic potential of other TLR-related markers (e.g., TLR1, TLR2, TLR4, TLR6, TLR10) in relation to aggressive colorectal cancer. This article will lead the development of the next generation of TLR-targeted therapeutics for the effective treatment of aggressive colorectal cancer.

Keywords: Colo-rectal cancer; Inflammation; Toll-like receptor; Innate immunity; Preclinical studies; Clinical trials; Genetic variants

Introduction

Globally, approximately 1.4 million people are diagnosed with colorectal cancer (CRC) and this disease has the highest morality rate compared to other gastrointestinal malignancies. Surgical resection is a relatively successful treatment against localized colorectal tumors, as evidenced by 90% five-year survival rates [1]. However, less responsive metastatic disease significantly reduces survival to 14% [1]. Moreover, disease recurrence rates are 22.5% to 82% among patients with pre-metastatic or metastatic disease who receive combination therapy (e.g., surgery, chemotherapy and or heat) [2]. Disease relapse and ultimately death following treatment of colorectal cancer occur, in part, because radiation, chemotherapy and even targeted therapies may lose their anti-carcinogenic and immune boosting capacity. Consequently, new therapeutic targets are needed to improve the efficacy of conventional treatment strategies, reduce recurrence and improve survival following treatment of colorectal cancer. Ideal therapeutic targets include innate immune-related signaling markers that play a vital role in tumorigenesis. Several studies suggest TLR-associated genetic susceptibilities are linked to alterations in aggressive colorectal cancer phenotypes [3-7]. Selected Toll-like receptor sequence variants alter TLR signaling to responsivity/interaction with pathogens, receptor dimerization or interaction, TLR-adapter protein interaction/communication, TLR signaling/expression, activation of interferon regulatory factors, cytokine secretion (IL-6, IL-8), cellular morphology (actin cytoskeletal disorganization, mitotic abnormalities), DNA repair capacity, epithelial-mesenchymal transition (Snail2, Vimentin), WNT signaling, cellular invasion, and inflammatory/immune responses favoring tumor growth. Repression or stimulation of innate immune-related markers may enhance the anti-carcinogenic and immune boosting capacity of conventional treatment strategies and improve disease prognosis following mono or combinational therapies. The current review will evaluate the TLR signaling pathway [8] in relation to colorectal outcomes from genetic epidemiology, pre-clinical and clinical studies. It will also shed light on immune boosting and anti-carcinogenic effects of TLR agonists alone or combined with radiation/chemotherapy against colorectal cancer under pre-clinical conditions. Lastly, this report will highlight gaps in the literature that require further investigation to expand options for the effective treatment of aggressive colo-rectal cancer.

Role of inflammation in colorectal cancer

Chronic intestinal inflammation plays a key role in the pathogenesis of colorectal carcinoma [9-13]. In fact, chronic inflammation of the gastrointestinal tract leads to the development of inflammatory bowel disease (IBD), including colitis that primarily presents as Crohn's disease (CD) or ulcerative colitis (UC). UC patients have persistent mucosal inflammation that can lead to the formation ulcers in the colon and rectum [14]. Whereas, Crohn's disease is characterized by inflammation of the digestive tract that spreads to the small or large intestine. These chronic inflammatory conditions can promote cellular insults and compensatory mechanisms leading to the development of colorectal cancer and other gastrointestinal-associated malignancies.

The intricate interplay between cellular extrinsic and intrinsic processes within inflammatory signaling greatly influences colorectal cancer (CRC). These processes include destabilization of genomic profiles, cellular proliferation/survival signaling, as well as alterations in the stromal environment and expression of epithelial-mesenchymal transition markers [15]. Recent research has identified the primary inflammation-related mechanisms involved in the initiation of

***Corresponding author:** LaCreis Renee Kidd, Department of Pharmacology and Toxicology, James Graham Brown Cancer Center, Louisville, KY, USA; Tel: +502/852-3465; E-mail: lrkidd01@louisville.edu

Received May 24, 2019; **Accepted** July 10, 2019; **Published** July 17, 2019

Citation: Reed DZ, Krupp N, Suman S, Yeyeodu S, Kimbro KS, et al. (2019) An Evaluation of Innate Immune Anomalies in Colorectal Tumorigenesis and Precision Medicine. J Cancer Sci Ther 11: 213-223.

colorectal cancer, including cellular proliferation, tumor escape of cell death, genomic instability, cellular invasion, and metastasis. For IBD, pro-inflammatory mediators (i.e., cytokines, chemokines), immune, epithelial and stromal cells interact together in the gut microenvironment to support neoplastic transformation. Similar to the tumor microenvironment, elevated cytokine (TNFα, ILs-1, 6, 12, 13, 17, 22 and 23) levels in IBD induce immune response regulators to promote colitis-associated tumorigenesis. In particular, pro-inflammatory cytokines induce the production of growth factors (e.g. TGF-β) and reactive nitrogen/oxygen species to direct damage to the colonic epithelium. This damage is consistent with high expression of COX-2 and NOS-2, inflammation-associated genes as well as STAT3 signaling activation [15,16]. Overall, genomic instability and neoplastic promoting mechanisms in IBD contribute to intestinal tumorigenesis.

Role of innate immune signaling in colorectal cancer

A vast array of regulators participate in inflammatory-induced CRC such as TLRs, their downstream signaling components and tumor suppressor proteins, proteolytic enzymes, nitric oxide synthase and cyclooxygenase family members, growth factors and cytokines [17]. In particular, cytokines and growth factors [transforming growth factors (TGFs)`, epidermal growth factors (EGFs) and vascular endothelial growth factors (VEGFs)] serve as mediators and end-products of TLR signaling. TLRs have counter-balancing roles in relation to cancer, as reviewed elsewhere [18-21]. TLR expression in innate and adaptive immune cells detected in healthy tissue supports anti-tumor immunity. Whereas, tumor-associated TLRs mediate resistance to apoptosis, tissue regeneration, stem cell activation, and recruitment of immune and stromal cells to the tumor's microenvironment, which supports tumorigenesis [18-21]. This review will primarily focus on the role of TLRs and selected downstream signaling markers in CRC tumor progression [17,22-26].

Cell surface Toll-like receptors: TLR1, TLR2, TLR4, TLR5, TLR6

The controversy surrounding TLR2's role in CRC, in part stems from the complex interaction between the colorectal epithelium, gut microflora and development of inflammatory conditions that precede CRC [27]. The commensal gut microbiota composition is a key factor that influences the functionality of TLRs in CRC [28,29]. For instance, Arditi and colleagues (2010) revealed $TLR2^{-/-}$ mice developed, dysregulated proliferation, a higher number of polyps and larger tumor volume in the colonic epithelia with evidence of inflammation (i.e., higher IL-6, IL-17A and STAT3 levels) and dysregulated proliferation compared to wild-type mice [30]. TLR2 knockout mice tumors showed less cell death and suppressed senescence [18], suggesting TLR activation may influence colon cancer in humans [31]. However in another study, no differences were observed in either colitis or polyp formation in $TLR2^{-/-}$ and wild type mice [32]. It is plausible these opposite findings are attributed to differences in the colonies' commensal gut microbiota despite shared genetics. Notably, gut inflammation levels are altered by the composition and/or total number of gut microbes following TLR2 inhibition, microbial transplantation, or antibiotic treatment [33].

TLR2 and TLR1/2 co-expression levels tend to increase during chronic inflammation and early stages of tumorigenesis [34]. However, TLR2 and TLR4 gene and protein levels in CRC tissue vary, presumably due to the geographic location and ethnic distribution of the study cohorts. In fact, a Japanese study (n = 50 cases) demonstrated TLR2 mRNA and protein expression were highly elevated relative to TLR4 in sporadic CRC relative to matched normal epithelia [31]. Moreover, average TLR2 expression in CRC tissue peaked in stage II patients; whereas, TLR2 elevated levels in control tissue demonstrated a TLR2-mediated inflammation in the epithelium of stage III patients. Higher mRNA levels of TLRs 1, 2, 4 and 8 were detected in 26 Taiwanese CRC patient tumors compared to adjacent normal tissue [34]. TLR2 and TLR4 positive cells via immunohistochemistry were predominantly located in tumor infiltrating lymphocytes; whereas, TLR1 and TLR8 overexpression was evident in tumor tissue. Notably, a comparison of both studies revealed a wide variation in TLR1 and TLR2 expression in the normal tissue collected from Japanese and/or Taiwanese study participants [31,34]. In contrast, TLR4 but not TLR2 mRNA was expressed in primary CRC cells, but not matched control colon epithelial cells from five European patients [35].

TLR4 dysregulation in CRC plays an intricate role in disease progression. Several studies demonstrate TLR4 up-regulation in CRC tissue when compared to normal (adjacent) tissue [34,36-40]. For instance, Xu and co-workers (2014) observed a 53% fold increase in TLR4 levels in colon tissue (n = 60) relative to normal adjacent (n = 20) tissue collected from Chinese patients [9]. Moreover, Santaolalla and co-workers (2015) demonstrated 38% of sporadic human colorectal cancers (n = 52) over-expressed TLR4 compared to 8% of normal tissue (n = 12) in human tissue microarrays [38]. TLR4 upregulation is related to increased epithelial proliferation, longer colonic crypts, expansion of Lgr5 crypt cells as well as an increased number of colonic tumor in azoymethane-induced CRC transgenic mice when compared to controls [38]. Other studies observed high TLR4 levels corresponded with poor disease prognosis, including poorly/moderately differentiated tumors (Dukes stages B-D vs. Stage A), tumor size (> 5cm; $p = 0.003$), advance tumor stage (TNM stage > III; $p = 0.017$), high tumor grade ($p = 0.002$), lymph node metastasis ($p < 0.001$) and distant metastasis ($p = 0.003$), [9,34,37,41-43]. Sussman and co-workers (2014) observed higher levels of TLR4 in the stroma and epithelial tissue from CRC patients with stages II (n = 61), III (n = 72) and IV (n = 25) relative to stage I (n = 24) disease [41]. There are mixed findings on the relationship between TLR4 and poor disease prognosis [9,40-42,44-51]. TLR4 was marginally linked to poor survival among 108 Japanese CRC patients. However, this relationship may become significant among high expressors of both TLR4 and its downstream marker (MyD88) [40]. Notably, high expression of TLR4 combined with MYD88 was significantly related to 5-year disease-free survival (DFS) (HR = 2.06; 95%CI = 1.11-3.82; $p = 0.026$) and overall survival (OS) (HR = 2.4; 95%CI = 1.28-4.52; $p = 0.0041$), after adjusting for histology, TNM stage, as well as vascular and lymphatic invasion [40].

Observed TLR4 and CRC outcome relationships may also depend on whether TLR4 was measured in fibroblast or tumor cells [42]. Notably, Eiro and co-workers (2013) demonstrated TLR4 expression by tumor cells was linked with a lower rate of CRC recurrence [42]. However, in the same study, TLR4 expression in fibroblasts was associated with a high tumor recurrence rate and shortened overall survival among patients with left-sided colon and rectal cancer [42]. Thus, the role of TLR4 in CRC is linked to its cross talk with downstream markers as well as its expression in tumor cells or inflammatory/immune cells in the stroma.

The massive effect of TLR4 on aggressive colorectal cancer phenotypes is related to the stimulation of TLR4 alone or the TLR4/MD2 complex by TLR4's ligand (i.e., LPS). In fact, LPS stimulation of the TLR4/MD2 axis resulted in an up-regulation of CXCR7, cellular proliferation and migration of human SW480 and colo205 colorectal carcinoma cell lines treated with exogenous CXCL12 [9]. However, additional studies are needed to elucidate the mechanism by

which TLR4/MD2 signaling stimulates CXCR7 expression. Zhu and colleagues (2016) showed LPS plays a role in cell migration, invasion, lymphangiogenesis and lymph node metastasis by inducing VEGF-C, a lymphangiogenesis and lymph node metastasis regulator via TLR4-NFkB/JNK signaling. In this study, TLR4 stimulation increased Human Dermal Lymphatic Endothelial Cells' (HDLECs) tube-like formation *in vitro*. Moreover, LPS accelerated lymphangiogenesis and lymph node metastasis in nude mice, presumably due to elevated VEGF-C [52,53]. These LPS induced effects on cancer cell motility and HDLECs were abrogated via VEGF inhibition via NFκβ/JNK signaling [52].

Although there is a debate as to whether or not LPS increases TLR4 expression, a few studies suggest LPS stimulation of TLR4 increases NFκβ signaling and pro-inflammatory cytokine (IL-6, IL-8) production [37,52,53]. Consequently, it is speculated LPS activation of TLR4/NFkB signaling in colon cancer leads to enhanced production of pro-inflammatory cytokines (IL-6, IL-8) that corresponds with high tumor grade/stage and colitis associated colon cancer (a CRC precursor), lymphangiogenesis, tumor growth, metastasis, and disease recurrence [34,37,53,54]. Once these cytokines bind to their respective receptors, they activate gp130βR, which triggers various signaling pathways (e.g., JAK/STAT) responsible for the up-regulation of genes involved in cell cycle progression, cell survival, angiogenesis and inflammation [55].

LPS-induced TLR4 signaling also activates a number of kinases, including p38 MAPK, p42/44 (ERK1/2) MAPK and AKT in colorectal and/or colon carcinoma cell types [39,43,56-58]. LPS treatment triggers AKT activation, but not p38 and p42/44 (ERK1/2) in metastatic CRC cells expressing TLR4 (HT-29 cells) [43]. In the same study, Hsu and co-workers (2011) demonstrated CRC cells that expressed TLR4/MD2 had enhanced liver metastasis after intrasplenic graft in immunocompromised nude mice [43]. They also observed adherence of metastatic CRC cells to different extracellular matrices and human umbilical vein endothelial cells (HUVEC) relative to non-metastatic cells was induced by LPS in the absence of TLR4 expression. However, this adherence was abrogated by a TLR4 antagonist, PI3K inhibitor, and anti-B1 integrin blocking antibodies. Collectively, these findings suggest LPS stimulation of the TLR4/MD2 complex activates PI3K/AKT signaling and promotes downstream B1 integrin function, which in turn increases the adhesiveness and metastatic potential of CRC cells. Alternatively, LPS stimulation of TLR4-NFkB signaling increases cell adhesion *in vitro* by LPS-NOX1 redox signaling [59]. O'Leary and co-workers (2012) demonstrated LPS activation of TLR4-NFkB increased NOX enzyme generation of ROS in colon cells (SW480, SW620, CT-26). LPS activation of NOX-ROS corresponds with an increase in PI3/Akt and a subsequent increase in cell adhesion to collagen in SW480 cells [59]. Tang and co-workers (2010) observed LPS stimulation of TLR4-NFkB signaling proteins attenuates TRAIL induced apoptosis in SW480 [53]. This suggests NFkB activation is essential in immune surveillance and cell death evasion of colon cancer cells.

Innate immunity regulator TLR5 plays a critical role in several biological and inflammatory components, including the gut epithelium, macrophages, dendritic cells (DCs) and T-cells. TLR5 undergoes activation after it binds to flagellin, the major protein detected in invasive flagellated bacteria at the mucosal surface of the gut [27,60]. TLR5 activation promotes IL-17 and IL-22 mediated early defense against pathogenic invasion in host tissue via the following adaptive immune responses: differentiation of naive B-cells into plasma cells, leading to IgA production; promotion of antigen-specific Th1 and Th17 cell development [27,61]; and mucosal production of interleukins (i.e., IL-17, IL-22). Furthermore, TLR5 activation inhibits the generation of regulatory T-cells (Tregs) but supports effector T-cell propagation. TLR5 over-expressing DCs in *lamina propria* induce effector T-cell responses against flagellated pathogens; whereas, lower TLR5 levels maintain homeostasis via Tregs induction.

TLR5 recognition of flagellin is the primary intestinal epithelium mechanism that induces an inflammatory response against infections, such as *Salmonella enteric* [27,62]. TLR5 knockout (TLR5 KO) mice remain resistant to *Salmonella* infection, presumably due to phenotypic adaptations in the small intestine and colon. These adaptations include up-regulation of host defense genes that regulate innate and adaptive immunity, such as antimicrobial peptides, serum and fecal IgA and IgG and gut transport proteins [62,63]. As a result, a homeostatic shift occurs in the microbiota composition of TLR5 KO mice, including an increase in E. coli and other enterobacterial species [62,64]. This increase corresponds with higher E. coli levels in CRC patients compared to healthy subjects, as previously mentioned [65,66].

Since intestinal epithelial cells communicate with gut microbes via pattern recognition receptors, the physiological consequences of host-commensal interactions in intestinal epithelial cells influence tumor development and progression in the gut [67]. However, to our knowledge, only one study has reported on the role of TLR5 in colorectal cancer. Rhee and co-workers (2008) reported TLR5-knockout (TLR5-KO) human colon cancer cells (DLD-1) in nude mice led to increased tumor volume/weight, reduced tumor necrosis and decreased reduction in neutrophil specific infiltration markers (7/4, Gr-1) in tumors relative to wildtype mice [67]. On the contrary, flagellin induced TLR5 signaling resulted in anti-tumor activity. Collectively, these findings suggest TLR5 stimulation may serve as an ideal immunotherapeutic approach to reduce colon tumor development. However, additional studies are needed to determine whether TLR5-dependent signaling significantly alters intestinal tumorigenesis.

Nucleic acid-sensing Toll-like receptors: TLR3, TLR7, TLR8 and TLR9

ER-Golgi and endolysosomal membrane systems house all nucleic acid sensing (NAS) TLRs (TLR3, TLR7, TLR8, and TLR9). The trafficking patterns of NAS TLRs to the plasma membrane and intracellular spaces vary. In this regard, DNA recognition specific TLR9 is the most widely distributed among NAS TLRs, with substantive evidence of its presence in certain tissues, including the colon, lymph nodes, plasma membrane and outside the cell [68,69]. In contrast, TLR8 is expressed in most tissues, but its intracellular distribution is limited to the membrane systems common to all NAS TLRs [69]. Similar to TLR8, TLR3 and TLR7 are widely distributed among all tissues, but they are also present at the cell surface like TLR9 [68]. Although NAS TLR localization mechanisms are not fully understood, UNC93B1 facilitates the trafficking of TLR3 (but not other NAS TLRs) trafficking to the plasma membrane [70]. Furthermore, UNC93B1 increases the half-life of TLR3 (and to a lesser extent TLR9's) responsiveness of all NAS TLRs to agonists [71]. Moreover, TLR3 bridges both innate and adaptive immune responses as well as defends against viral infection by binding to dsRNA, activating NFkβ, and producing type I interferon [72,73]. However, there is some uncertainty on whether TLR3 is upregulated in CRC [74-76].

Limited studies have addressed the role of TLRs 7-10 in CRC. TLR 7-10 genes were upregulated in the tumor tissue of CRC patients [34,77]. However, only TLR8 expression correlated with poor prognosis among German CRC patients (n = 65) [77]. TLR9, localized in the endosomal compartment, recognizes intracellular bacteria by binding

unmethylated cytosine phosphate guanine (CpG) dinucleotides [27,78]. TLR9 engagement with CD4 T cells prolongs their survival and involvement in antitumor responses [79,80]. Recent reports offer some insight on the role of TLR9 signaling in colonic carcinogenesis. Moreover, TLR9 is down regulated in the hyperplastic and villous polyps of CRC patients. Thus, TLR9 expression is protects against malignant transformation of colorectal mucosa [81]. Moreover, TLR9 reduces apoptosis in gastrointestinal inflammatory disease.

Once activated TLR9 elicits either anti- or pro-inflammatory responses depending on whether its activation occurs on the apical or basolateral surface of the intestinal epithelium [27]. In experimental colitis models, the administration of CpG and other TLR9 agonists triggers apical TLR9-mediated tolerance, including a decrease inTLR4 induced pro-inflammatory cytokine signaling, apoptosis, production of IFN-gamma and IL-6, anti-inflammatory IL-10 elevation, and ultimately a reduction in disease severity of colitis as well as other intestinal diseases [27]. Notably, TLR9 activation can limit TLR4 signaling and subsequent inflammation and cell death in the gut [82]. On the contrary, basolateral TLR9 activation drives the production of pro-inflammatory cytokines (i.e., TNF, IL6, IL12) and NF-κB induced IL-8 secretion [27].

Another important effector of TLR signaling is MyD88. TLR/IL1R employs MyD88 as an adaptor to bridge inflammatory and RAS oncogenic signaling pathways [83]. Up-regulated MyD88 is required for Ras-dependent proliferative signaling and malignant transformation [83-85]. In fact, MYD88 repression increases cellular damage, followed by increased cell death and improved chemotherapeutic responsiveness to cisplatin against colon cancer using *in vitro* and murine xenograft colon models [86]. This anti-tumorigenic and enhanced chemotherapeutic effect is linked to MYD88's influence on RAS-dependent DNA repair and tumor suppressor genes, namely p53 and its target p21. In terms of DNA repair, MyD88 suppression decreases RAS activation of ERCC1 expression, leading to lowered nucleotide excision repair protein levels, increased DNA damage and subsequent increased cell death [87]. Alternatively, repression of MyD88 induces apoptosis through activation of p53 and its target p21 [83,86]. MyD88 repression reduces colon cancer proliferation and increases tumor cell death and sensitivity to cisplatin in colon cancer cell lines and murine xenograft models [83,86]. Notably, repression of cell models with RAS activation mutations or null for p53 fails to trigger apoptosis and presumably does not enhance chemotherapy responsiveness [83,86].

Innate immune signaling pathway sequence variants and colorectal cancer outcomes

Several studies suggest TLR-associated genetic susceptibilities are linked to alterations in colorectal cancer outcomes, including neoplasm, adenoma, colorectal cancer-specific death, poor differentiation, high UICC TNM stage, lymph node metastasis, and metastasis [3-7]. Selected Toll-like receptor sequence variants alter TLR signaling to responsivity/interaction with pathogens, receptor dimerization or interaction, TLR-adapter protein interaction/communication, TLR signaling, activation of interferon regulatory factors, cytokine secretion (IL-6, IL-8), cellular morphology (actin cytoskeletal disorganization, mitotic abnormalities), DNA repair capacity, epithelial-mesenchymal transition (Snail2, Vimentin), WNT signaling, cellular invasion, and inflammatory/immune responses favoring tumor growth.

Careful evaluation of genetic epidemiology data provides intriguing observations of the complex role of TLR2 in CRC. A Brazilian cohort revealed a functional *TLR2-196* to *-174del* polymorphism alters mRNA and protein expression as well as CRC susceptibility. Carriers of the *TLR2-196* to *-174del variant allele* had a 1.6-1.7-fold increase in CRC risk under the dominant (OR = 1.72; 95%CI = 1.03-2.89; p = 0.038) and additive (OR = 1.59; 95%CI = 1.02-2.48; p = 0.039) genetic models compared to the referent genotype [88]. Notably, carriers of the variant *TLR2-196* to *-174del* allele possessed higher levels of the TLR2 transcript (2.2 fold higher) and protein (p = 0.03) relative to those with the referent genotype. In contrast, Noguchi and co-workers (2004) demonstrated the del/del polymorphism is linked to decreased gene transcription within a Japanese population [89]. However, given the impact of this functional del/del SNP in other cancers, the *TLR2-196* to *-174del* polymorphism presumably enhances the pro-inflammatory cytokine production favoring tumorigenesis. In a small CRC study (193 cases, 278 controls), TLR2 rs3804099 $T^{597}C$ was linked to a 79-90% reduction in CRC risk among Portuguese patients, which remained significant when the analysis was restricted to women or non-smokers [90]. This TLR2 rs3804099 $T^{597}C$ synonymous SNP, may alter: RNA stability, which in turn may alter protein expression and function; or dysregulate timing of co-translational folding of the gene product that alters the interaction of the gene product with its substrates [91,92]. TLR2 rs3804099 is located in the coding sequence of the extracellular domain of TLR2 at residue 199, an N-linked glycosylation site. Consequently, this synonymous SNP may obscure or expose the N-glycosylation site, which may subsequently alter TLR2 trafficking, stability or signaling. Interestingly, monocytes with the TLR2 rs3804099 CC genotype was related to a 41% decrease in TNF-alpha production relative to the TT genotype [90]. Although TLR4 rs5743704 and rs5743708 were not linked to CRC, they were associated with CRC survival [93]. Possession of at least one TLR2 rs5743704 A (HR = 1.89; 95%CI = 1.26-2.83) or TLR2 rs5743708 A (HR = 1.74; 95%CI = 1.12-2.70) minor allele was linked to a 74-89% increase in overall colon cancer survival [93]. TLR2 rs5743704 is located at or near the Toll/interleukin-1 receptor (TIR) homology domain critical for the initiation of TLR signaling. Moreover, the TLR2 rs5743708 sequence variant is located at the carboxy terminal 19-amino acid ATG16L1-binding motif that is implicated in TLR2 signaling during phagosome formation in macrophages [94].

To our knowledge, there are two published reports on the relationship between colorectal cancer and sequence variants detected within intracellular TLRs, namely TLR3, TLR8 and TLR9 [3,93]. Castro and co-workers (2011) studied 614 German men and women diagnosed with colorectal cancer and revealed inheritance of the TLR3 rs3775291 TT genotype (HR=1.93; 95% CI = 1.14-3.28) was linked with a 93% increase in CRC-specific death relative to patients with the referent CC genotype [3]. The relationship persisted even after adjusting for age or clinico-pathological parameters. The TLR3 rs3775291 Leu412Phe polymorphism may disrupt the translation of the TLR3 protein leading to either a miscommunication or no interaction with its adapter protein, TRIF. Furthermore, this non-synonymous SNP may disrupt TLR3 signaling, block TLR3's role as an anticancer immune stimulator and allow the tumor cell to escape cell. Slattery and co-workers (2012) evaluated four TLR3 SNPs (rs5743305, rs11721827, rs3775292, rs3775291) in relation to colon (1,555 cases, 1,956 crtls) and rectal cancer 754 cases, 959 crtls) susceptibility in a multi-ethnic study (90% Caucasian, 5% African-American, 5% Hispanic) [93]. Among these, TLR3 rs11721827 was linked to a 27% increase in rectal cancer risk under the dominant genetic model to a 27% increase in rectal cancer risk; whereas, the TLR3 rs3775292 sequence variant was associated with a 32% decrease in colon cancer risk under the

recessive genetic model. TLR3 rs11721827 and TLR3 rs3775292 are both intronic SNPs that may alter regulation of TLR3 transcription. Unfortunately, polymorphisms detected in other intracellular TLRs, such as TLR8 (rs3761623 Promoter) and TLR9 (rs352140 P545P, rs5743836 Promoter) did not significantly modify colorectal cancer outcomes [3].

Selected TLR genetic alterations are linked with colorectal cancer risk, disease progression, and poor prognosis, albeit unequivocally. The TLR4 rs4986791 (T399I or 1196 C>T) is a non-synonymous SNP predicted to have a possible damaging effect on the TLR4 protein. The variant 399 T allele is linked to lower gene expression or conformational change of the recognition site with PAMPs [95]. This functional change may lead to a decreased induction of innate and adaptive immunity mediators or aberrant STAT3 activation and subsequent induction of cancer related genes. Collectively, the biological consequences of this SNP may contribute to increased CRC risk and poor disease prognosis. In a pilot study, Omrane and associates (2014) revealed inheritance of the TLR4 rs4986791 CT genotype was associated with a modest increase in the risk of poor colorectal tumor differentiation (OR = 1.12; 95% CI = 1.02-1.22), lymph node metastasis (OR = 1.31; 95% CI = 1.02-1.69) and metastasis (OR = 1.5; 95% CI = 1.02-2.25) among 240 Tunisian men (53 cases, 45 crtls) and women (47 cases, 95 crtls) [4]. In the same study, the $T^{399}I$ polymorphism was linked to tumor differentiation ($p = 0.027$) and tumor architecture ($p = 0.02$) among colorectal cancer patients [4]. However, five studies did not reveal a significant difference in the distribution of the possession of the TLR4 rs4986791 variant T allele between colorectal cancer cases and controls among German, Croatian, Malaysian, and Russian patients [93,96-99]. Despite these null reports, two meta-analyses demonstrated higher CRC susceptibility among carriers of the TLR4 rs4986791 variant allele [100,101]. In a 2014 meta-analysis of six studies (1,209 cases, 1,218 crtls), the TLR4 rs4986791 was related to a 1.3-1.77 fold increase in the risk of developing colorectal cancer under the additive and dominant genetic models [99-101]. In another meta-analysis, including six observational studies (619 cases, 632 crtls), harborers of the TLR4 rs4986791 ^{399}TT and TC+CC genotypes had a 4.5-4.99 fold increase in CRC risk compared to those with the CC genotype [97-101]. Additional studies in larger and racially diverse sub-group may help clarify the role of TLR4 rs4986791 in CRC.

Several independent studies and two meta-analyses evaluated the relationship between colorectal cancer and the inheritance of TLR4 rs4986790 $D^{299}G$, which has the same functional consequence as the TLR4 rs4986791 T399I SNP [4,88,90,96-105]. Nine studies and two-meta-analyses did not reveal significant differences in the frequency of the variant TLR4 rs4986790 allele comparing and colorectal cancer cases and controls among participants of European, Asian and Hispanic descent. On the contrary, three studies demonstrated the TLR4 rs4986790 variant G allele linked to a 1.18-2.25 fold increase in colorectal cancer among Croatians, rectal cancer and Russians as well as advanced and metastatic disease among Tunisians [4,97,98]. Similarly, German CRC cases (n =- 214) who possessed the TLR4 rs4986790 ($D^{299}G$) variant allele were more likely to have advanced disease (UICC ≥ stage 3; 70% vs 46%, $p = 0.142$) and evidence of metastasis; 42% vs 19%, $p = 0.0065$) relative to those with the referent genotype [96]. In the same study, stably expressing TLR4 rs4986790 $D^{299}G$ Caco-2 cells displayed: a fibroblast-like appearance; higher levels of cellular proliferation and invasion; elevation in EMT markers (Snail2, Vimentin), cytoskeletal disorganization (i.e., Cx43, DKK1), and mitotic abnormalities relative to TLR4 WT Caco-2 cells. Additionally, intestinal xenograft tumors from Caco-2 TLR4 $D^{299}G$ cells in CD1 nu/nu female mice exhibited rapid growth after 22 days compared to Caco-2 TLR4 WT xenografts. This accelerated tumor growth may be attributed to the activation of an innate immune-related gene (i.e., phosphorylated STAT3) via Wnt signaling. Notably, TLR4 $D^{299}G$ Caco-2 cells treated with a STAT3 inhibitor (100μM ofNSC74859 or anti-oxidant agent (i.e., 15 uM quercetin) resulted in a significant reduction in phosphorylated STAT3 protein, cellular invasion and tumor growth relative to vehicle control.

A few investigators reported on the impact of other TLR4 sequence variants on colorectal cancer risk [93,102,103,106]. Koop and co-workers (2015) reported carriers of the TLR4 rs5030729 AA genotype had a 1.3 fold higher risk of developing CRC (IRR = 1.3; 95%CI = 1.06-1.61) when compared to those with the TT genotype among Danish participants of a prospective case control study [102]. In the Clue II study, mentioned earlier, Tsilidis and co-workers (2009) demonstrated individuals who possessed the C allele for the TLR4 rs7873784 G>C SNP, located in the 3' UTR, was linked to a 42% reduction in CRC risk (OR = 0.58; 95%CI = 0.37-0.91) relative to the reference genotype [103]. Contrary to the null findings in the Clue II study, Slattery and associates (2015) reported inheritance of the TLR4 rs11536898 AA genotype was inversely related to colon cancer (OR = 0.5; 95%CI = 0.29-0.87) but not rectal cancer [93]. However, this significance was lost after adjusting for multiple hypothesis testing. Within the aforementioned studies, several TLR4 SNPs (rs11536898, rs11536889, rs2737190, rs10116253, rs1927914, rs1927911, rs2149356, rs11536891, rs11536891, rs11536898, rs10759932, rs5030728, rs12377632, rs1554973, TLR4 -160 T/C, rs1927907, rs11536879, rs7873784), were not related to CRC risk among European American, Danish and Brazilian men [88,93,103,106].

TLR-associated sequence variants in the transmembrane receptor, TLR5, have both protective and stimulating effects on poor disease prognosis. Klimosch and associates (2013) evaluated two TLR5 sequence variants (rs2072493 $N^{592}S$; rs5744174 $F^{616}L$) in relation to colorectal cancer risk among 613 German patients [5]. Possession of the TLR5 rs2072493 AG+GG genotype was strongly associated with a 1.6-1.9 fold increase in colorectal cancer-specific mortality (HR = 1.89; 95% CI = 1.27-2.80) and overall mortality (HR = 1.57; 95% CI = 1.08-2.27) [5]. However, possession of at least one TLR5 rs5744174 C variant allele was linked to a 34-49% reduction in colorectal cancer death and lymph node metastasis (OR = 0.59; 95%CI = 0.36-0.95; CC vs TT) Upon stratification by disease site, the findings for both the TLR5 rs2072493 and rs5744174 sequence variants were only statistically significant among patients who had colon cancer. Moreover, the TLR5 rs2072493 $N^{592}S$ SNP was related to distant metastasis (OR = 1.81; 95% CI = 1.13-2.90) and low tumor stage under the dominant genetic model (OR = 1.52; 95% CI = 1.06-2.20). According to structural evidence and modeling studies, the two TLR5 SNPs are not in direct contact with TLR's activation site (i.e., flagellin D1) but they may alter the TLR5's dimerization or interaction with the flexible flagellin D_0 domain. Given the role of TLR5 in the gut and colorectal cancer, the investigators assessed whether these to sequence variants would modify TLR5 signaling. Interestingly, these two TLR5 SNPs have opposing effects in TLR signaling using kidney 293T cells stably transfected with a single copy of TLR5 WT or variants. Consequently, the TLR5 rs2072493 $N^{592}S$ variant allele revealed a slight increase in IL-8 levels and TNF mRNA induction upon stimulation with S. typhimurium flagellin relative to the wildtype. However, these same cytokines were reduced in cells with the rs5744174 $F^{616}L$ relative to the referent genotype. Additional studies revealed the TLR5 rs5744174 $F^{616}L$ variant responded poorly toward all bacterial species tested; whereas, the TLR5 rs2072493 $N^{592}S$ variant revealed differential functional modulation depending on the

bacterial species. The TLR5 rs2072493 $N^{592}S$ demonstrated reduced responsivity to *E. coli* and *P. Vulgaris* compared to the WT. However, responsivity to gut-resident E. cloacae, A. faecalis and P. mirabilis were equally high for the TLR5 $N^{592}S$ and WT. Neither of these two TLR5 SNPs modified TLR5 functional activity in HCT116 and DLD1 colorectal cells. However, the TLR5 rs5744174 $F^{616}L$ variant was hyporesponsive to the TLR5 flagellin ligand but not TLR5 R848 ligand in monocytes as demonstrated by a reduction in phospho-p38 and CD62L shedding relative to the WT genotype. Additional functional studies should assess whether these TLR5 sequence variants have functional consequences in other colorectal cancer cell lines and blood immune cells collected from racially/ ethnically yh diverse subgroups. Given the epidemiological and *in vitro* evidence, these two SNPs may play a significant role in innate immunity, immune surveillance and CRC progression.

Impact of innate immune signaling suppression or stimulation on colorectal cancer treatment

Colorectal cancer patients undergo treatment with surgery, radiation therapy, chemotherapy, or targeted therapy. However, these treatment strategies may lose their anti-tumorigenic and or immune enhancing effects due to an acquired drug resistance, up-regulation of oncogenic pathways and compromised DNA repair capacity. Manipulation of the TLR signaling pathway may potentiate the immune boosting and anti-carcinogenic effects of radiation, chemotherapy or targeted therapy. Recent use of TLR agonists or antagonists in pre-clinical and clinical trials stems from (Supplemental Tables 1 and 2), in part, the role these innate immune manipulators play in tumor growth, cellular proliferation, cell death, inflammatory/immune responses, and DNA damage.

Although TLRs are mainly expressed in innate immune cells (e.g., dendritic cells (DCs) they are also detected in tumor cells. The presence of TLRs on the cell surface of cancer cells suggests they may contribute to tumorigenesis and/or sensitivity toward chemopreventive agents. One study demonstrated TLR4 signaling and activation are enhanced in CRC cell models [107,108]. Pham and colleagues (2010) revealed a TLR4 agonist improved cyclophosphamide cytotoxicity by enhancing adaptive immune response activity [109]. The TLR4 agonist lipopolysaccharide significantly enhanced DC maturation via natural killer (NK) cells in co-cultures [109]. TLR4 stimulation activates natural killer (NK) cells to stimulate immature DCs [109]. The interaction between NK cells and DCs results in activation and cytokine production in both cell types [109]. The addition of a TLR4 agonist also increased production of cytokines (i.e., IL12-p40 and IL12p70) [109]. Further analyses demonstrated the addition of a TLR4 agonist and IL2 exposure to both NK cells and DCs stimulated T cells in a dose-dependent manner using allogeneic models [109]. Notably, IL2 is a cytokine known to activate NK cells, which in turn further increases cytokine production [109]. This boost in immune cell response along the TLR4-IL2 axis was associated with improved antitumor activity when combined with cyclophosphamide in CT-26 murine colon cancer models [109].

Nucleic acid sensing (NAS) TLRs (TLR3, TLR7, TLR8 and TLR9) are promising therapeutic targets, as nucleic acid analogs for these TLRs are currently in clinical trials for the treatment of a variety of malignancies, including colorectal cancer [110,111]. Furthermore, the development of the next generation of NAS TLR-targeted therapeutics is an active area of research [110,112,113]. TLR ligands exert anti-tumor effects against colorectal cancer by inducing cell death or activating immune cells [108]. Stier and colleagues (2013) showed TLR3 (Poly I:C), TLR4 (LPS), TLR7 and TLR8 (R848) ligands as single agents or in combination with Taxol, did not possess significant anti-proliferative effects against CRC cells [108]. However, these ligands stimulated immune cells [i.e., T cells and natural killer (NK) cells] in a dose-dependent manner, which in turn increased cytotoxicity against CRC cells [108]. Although the addition of TLR3, TLR4, TLR7 and TLR8 ligands to Taxol did not enhance tumor cell killing *in vitro*, combinations of Taxol+TLR7/8 ligands inhibited tumor growth *in vivo* [108]. Furthermore, the investigators observed greater anti-tumor effects using treatment combinations along with lymphocytes *in vitro* [108]. Treatment of colon tumor-bearing mice with a dual agonist of TLR7 and TLR8 elicited a MyD88-dependent antitumor response, substantiating the value of developing NAS TLR therapeutics and identifying the critical role of MyD88 in TLR7- and TLR8-mediated anti-tumor immunity [114]. Interestingly, there is a TLR3 agonist poly-ICLC clinical trial combined with CTLA-4 antibody and PD-L1 antibody treat multiple types of solid tumors, including colorectal cancer (NCT02643303).

The TLR3 ligand polycytidylic acid (Poly I:C), a synthetic form of dsRNA, reduced cell proliferation as well as increased nuclear apoptosis and NFkB activity in Poly I:C transfected SW480 and/or Colo320 colon carcinoma cell lines [74]. Since TLR3 can trigger apoptosis in tumor cells, Taura and co-workers (2010) hypothesized TLR3 stimulation with 5-flurouracil (F-FU), a P53 activating P53 compound would mediate TLR-3 related tumor cell killing [115]. As a consequence, the investigators revealed 5-FU increased TLR3 levels as well as coupled increased TLR3-related cell death in P53 expressing colon carcinoma cell lines (HCT116 $p53^{+/+}$) transfected with Poly I:C. This increase in apoptosis was more pronounced in P53 expressing colon carcinoma cells when compared to P53 null cells.

In murine models, 5-fluorouracil (5-FU) combined with the TLR5 agonist Entolimod reduced toxicity to 5-FU, a chemotherapeutic agent often used to treat patients with metastatic CRC. Resistance to 5-FU is linked to upregulation of genes associated with apoptotic inhibition and cellular stress/defense, including IRAK1, MALTI, BIRC5, MICB, and SDF2L1 [116]. The protective effects of Entolimod are mediated through NFκB induction of IL6 [117]. Notably, a Phase I TLR5 agonist Entolimod clinical trial involved patients with unspecified solid tumors; however, it is not clear whether this study consisted of colorectal cancer patients (NCT01527136).

Systemic delivery of small molecule TLR7 agonists can prime immune system responses to reduce tumor and metastatic burden [118]. TLR7 agonist DSR-6434 displays significant anti-tumor activity in BALBc and C57B16 murine models [118]. DSR-6434 boosts IFNγ and CD8+ T cells, suggesting this agent is capable of producing a long-lasting immune response [118]. Furthermore, exposure to 15 Gy ionizing radiation (IR) and DSR-6434 (0.1 mg/kg) generated synergistic effects, which surpassed tumor growth reduction caused by either therapy alone [118]. Also, local IR combined with DSR-6434 reduced lung metastasis incidence and improved survival in the metastatic sarcoma KHT model [118]. In a nutshell, the TLR7 agonist DSR-6434 potentiates and elongates the anti-tumorigenic effects of ionizing radiation (e.g., inducing DNA damage, triggering cell death, stimulating immune cell activation, activating antigen-presenting cells, releasing immunosuppressive cytokines) [118]. Perhaps this enhanced anti-tumorigenic response is attributed to monocyte-derived IL6 activation of NK cells and CD8+ T cells [118].

The TLR7 agonist imiquimod (IMQ) exhibits both anti-cancer and inflammatory activity [119]. Although this agent increases host

response, it can also stimulate immunosuppressive markers, such as iNOS, IL10, and indoleamine 2,3-dioxygenase (IDO) [119]. IMQ treatment also increased expression of IL2, IL12b, FasL, and CCL2 in IDO-KO mice and draining lymph nodes. Hence, in the absence of IDO, IMQ stimulates the release of cytokines needed for antigen-specific T cells [119]. TLR signaling can activate innate as well as adaptive immune cells. TLRs are primarily expressed on antigen presenting cells [i.e., dendritic cells (DC) and macrophages], but are also found on natural killer (NK) cells, B cells, and cytotoxic CD8+ T cells [120]. Antigen contact and TLR activation can lead to increased proliferation, cytokine secretion, co-stimulatory molecule expression, phagocytic activity, and antigen presentation in these immune cells [120]. More specifically, TLR7 activation can stimulate DCs to produce a pro-inflammatory response associated with increased cytokine release, antigen processing, and T cell activity [120].

TLR8 is a potent activator of innate immune response capable of stimulating Th1-polarizing cytokines and chemokines [121]. Consequently, Northfelt and co-workers (2014) revealed TLR8 agonist VTX-2337 rapidly increased G-CSF, IL6, MCP1, and MIP-1β in nonhuman primates [121]. A Phase I clinical trial demonstrated a high tolerance for VTX-2337 among cancer patients with primary and recurrent solid tumors, including colorectal, pancreatic, breast and renal cancers (NCT02650635). VTX-2337 also induced dose-dependent increases in cytokines and biomarkers of immune activation, such as GCSF, CCL2, CCL4, and TNFα. This agent exhibited the best overall anti-tumor response among patients diagnosed with stable disease [121]. Furthermore, administration of VTX-2337 as part of a combination regimen may enhance efficacy [121]. Additionally, investigators are evaluating VTX-2337 in a combination therapy in ovarian, Fallopian tube, and primary peritoneal cancers within a Phase 1/2 clinical trial (NCT02431559).

The TLR9 ligand, CpG oligodeoxynucleotide 1826 (CpG), inhibits colon adenocarcinoma tumor growth and liver metastasis in murine models via Th1 activity [122]. Th1 function undergoes enhancement by pro-inflammatory cytokines (e.g., IL-12, IL-18, IFNα, and TNFα), which stimulates NK cell activity and directly kills tumor cells with low MHC class I expression [122]. Upon binding to TLR9, CpG also regulates MHC class I and II molecule expression along with co-stimulatory molecules CD80 and CD86. Additionally, CpG stimulates the proliferation of B-cells, IgM secretion, and isotype switching [122]. The agonist also induces the infiltration of lymphocytes and macrophages in tumors; however, the exact mechanism is unknown [122]. TLR9, expressed in β-lymphocytes, monocytes, and plasmacytoid dendritic cells in humans, recognizes unmethylated CpG-ODN present in bacterial and viral DNA. Stimulation of TLR9 with CpG-ODN in combination with an anticancer drug (i.e., Adriamycin) reduces cell proliferation and viability as well as elevated NFkB activity in colon carcinoma cell lines (i.e., SW480 and/or Colo320 cells) [74]. Another TLR9 agonist, MGN1703, stimulates antitumor responses through NK cells, monocytes, macrophages, and cytokine induction [123]. MGN1703 is effective and well tolerated in CRC, renal cell, and melanoma patients. In a Phase II clinical trial study, MGN1703 demonstrated antitumor effects against CRC, including higher progression-free survival (PFS) in some patients, as well as increased activation of NK cells and monocytes [123]. Moreover, TLR9 agonists decrease estrogen activation in ER-positive breast cancer [81]. Since estrogen receptors have a functional role in colorectal cancer, TLR9 agonists may have therapeutic value in colorectal cancer by suppressing estrogen activation [81]. Collectively, these results suggest stimulation of TLR9 is pivotal for regulating cell viability, cytotoxicity of anticancer drugs, cell death and immune escape of tumor cells.

TLR9 agonists enhance antitumor and immune boosting effects of monoclonal antibodies and other agents targeting cancer growth pathways. Monoclonal antibodies (e.g., cetuximab and panitumumab) and receptor tyrosine kinase inhibitors (e.g., gefitinib and erlotinib) targeting EGFR are effective cancer treatments [124]. However, resistance is often a challenge with these agents. Resistance to tyrosine kinase inhibitors often occurs due to mutations or loss of EGFR, activation of other tyrosine kinases or signaling pathways, and induction of angiogenesis by tumor-derived factors [124]. In CRC and several other cancers, KRAS mutations can lead to activation of RAS/MAPK pathway signaling and subsequent resistance to EGFR inhibitors [124]. However, the TLR9 agonist, IMO, heightens the antitumor activity of anti-EGFR and anti-ERBB2 monoclonal antibodies by enhancing the immune effects of these agents [124]. IMO can boost immune response by increasing activation of NK cells, DCs, and cytotoxic T cells, as well as the production of antitumor cytokines [124]. IMO also enhances antibody-dependent (i.e., cetuximab) cell mediated cytotoxicity by inhibiting colorectal tumor growth and maintains sensitivity to cetuximab, based on *in vitro* and *in vivo* models [124].

Candidate TLR signaling targets ideal for precision medicine and colorectal cancer treatment

Prospective targets recommended for colorectal cancer therapy include TLR-associated targets that displayed a strong influence on CRC outcomes in the aforementioned epidemiological, pre-clinical and clinical studies. Candidate TLR-associated genes that may serve as potential therapeutic targets for the treatment of aggressive colorectal cancer include TLR3, TLR4, TLR5, TLR7, TLR8, TLR9, and MYD88. In particular, TLR signaling genes are upregulated (TLR4, TLR7-10) or downregulated (TLR9) in CRC tissue and/or inflammatory/immune regulatory cells in the stroma. Additional tumor-based studies are needed to assess levels of TLR2, TLR5, TLR6 and TLR10 in colorectal cancer tissue and the tumor microenvironment. TLR4 and TLR8 expression were linked with aggressive tumor behavior, including lymph node metastasis, metastasis, tumor recurrence, advance tumor stage, adenomas, large tumor size, large tumor volume, poor survival and disease recurrence. Stimulation to TLR (TLR3, TLR5, TLR7, TLR8, TLR9) ligands or MyD88 repression may exert anti-tumor effects and/or improve the efficacy of radiation/chemotherapy. For instance, the TLR3 agonist (Poly I:C) reduced cell proliferation and increased cell death in colon cancer cell lines. Apparently there is an on-going clinical trial to evaluate the impact of TLR3 agonist (Poly I:C) combined with a checkpoint inhibitor (e.g., CTLA-4). Improved effectiveness of conventional therapies against colorectal cancer are characterized by enhancing cell death of tumors, decreasing nucleotide excision DNA repair, stimulating adaptive immune response activity and enhancing tumor suppressor genes. Suppression of MyD88 *in vitro* enhances responsivity to chemotherapy, presumably through an increase in DNA damage, which in turn promotes cellular death of tumor cells. Thus several TLRs (TLR3, TLR5, TLR7, TLR8 and TLR9) and MYD88 are promising therapeutic targets for the treatment of a variety of cancers, including colorectal cancer. Additional studies will further elucidate the role of TLRs in colorectal cancer as well as assess how one or more agonist/antagonist improves the anti-tumor and immune boosting capacity of current therapies.

Collectively, the aforementioned functional SNPs suggest inheritance of high-risk TLR signaling related sequence variants

may help to identify patients who should receive more aggressive treatment for colorectal cancer. Alternatively, these high-risk sequence variants may also identify target genes that may undergo stimulation by TLR agonists to boost the immune system and improve response to available cancer treatments. Although there are no specific studies on the impact of TLR signaling associated SNPs on responsiveness to available therapies to treat colorectal cancer, studies involving breast cancer patients may offer some guidance [125-129]. In fact, non-metastatic breast cancer patients with lymph node involvement (n = 280) who possessed the TLR4 ^{299}Gly loss-of-function allele and received anthracycline chemotherapy and local radiotherapy relapsed more quickly after surgery, than patients who carried the TLR4 299Asp/Asp referent genotype. Five years post-treatment, the frequency of metastasis was significantly higher among *TLR4* 299Gly carriers (40%) relative to patients without the variant allele (26.5%) ($P < 0.05$, RR = 1.53; 95%CI = 1.1–3.58). Moreover, there was a lower percentage of metastasis-free patients with the mutated TLR4 when compared to those without inheritance of the variant allele ($P < 0.05$). Additionally, two copies of the TLR3 rs3775291 T variant allele decreased survival among colorectal cancer patients without adjuvant therapy [3]. Subsequent studies will help to assess whether genetic susceptibilities in TLR-related markers will alter responsivity to treatment with radiation, chemotherapy, immunotherapy (including immune stimulation with TLR agonists/antagonists) and combination therapy.

Discussion and Conclusion

Epidemiological, pre-clinical and clinical studies offer critical knowledge on the role of essential TLR signaling markers pivotal to colorectal cancer development and disease progression. Although genetic variations detected within the Toll-like receptor-related markers alter colorectal cancer outcomes and responsivity to adjuvant therapy, For instance, further research is needed to determine the functional consequence of various TLR-related sequence variants. In addition, many studies fail to include underserved populations (e.g., African Americans) who suffer disproportionately from colorectal cancer. Moreover, past and ongoing clinical trial studies shed light on TLR-associated therapeutic targets (TLR3, TLR4, TLR7-9, MyD88) that may help replace or complement conventional (i.e., immunotherapy, radiation, chemotherapy) treatment modalities against aggressive colorectal. TLR ligand stimulation or repression of downstream signaling markers (e.g., MyD88) may exert anti-tumor effects or improve the efficacy of radiation/chemotherapy. This improved effectiveness of conventional therapies against colorectal is characterized by enhancing cell death, decreasing nucleotide excision DNA repair, and stimulating adaptive immune response activity. Additional tumor-based, pre-clinical and clinical studies will further assess whether targeting one or multiple TLR markers will improve the efficacy of existing or new immunotherapy, radiation, chemotherapy modalities. Such efforts will help further develop of the next generation of cell surface and nucleic acid sensing TLR-targeted therapeutics for the effective treatment of aggressive colorectal cancer.

Acknowledgements

This research was supported in part by Bucks for Brains and the James Graham Brown Cancer Center.

Data Availability

All datasets generated for this study are included in the supplementary files.

Author Contributions

All authors discussed this project. SS generated the supplemental tables. DR, SY, NK, KK, LRK wrote the main text of the manuscript and all authors reviewed and intellectually contributed to the final draft of the manuscript.

Conflict of Interest Statement

The authors declare research was not conducted in the presence of any personal, professional or financial relationships that could potentially be construed as a conflict of interest.

References

1. American Cancer Society (2017) Colorectal cancer facts & figures 2017-2019.
2. Van Oudheusden TR, Nienhuijs SW, Luyer MD, Nieuwenhuijzen GA, Lemmens VE, et al. (2015) Incidence and treatment of recurrent disease after cytoreductive surgery and intraperitoneal chemotherapy for peritoneally metastasized colorectal cancer: A systematic review. Eur J Surg Oncol 41: 1269-1277.
3. Castro FA, Forsti A, Buch S, Kalthoff H, Krauss C, et al. (2011) TLR-3 polymorphism is an independent prognostic marker for stage II colorectal cancer. Eur J Cancer 47: 1203-1210.
4. Omrane I, Baroudi O, Kourda N, Bignon YJ, Uhrhammer N, et al. (2014) Positive link between variant toll-like receptor 4 (Asp299Gly and Thr399Ile) and colorectal cancer patients with advanced stage and lymph node metastasis. Tumour Biol 35: 545-551.
5. Klimosch SN, Forsti A, Eckert J, Knezevic J, Bevier M, et al. (2013) Functional TLR5 genetic variants affect human colorectal cancer survival. Cancer Res 73: 7232-7242.
6. Chang CM, Chia VM, Gunter MJ, Zanetti KA, Ryan BM, et al. (2013) Innate immunity gene polymorphisms and the risk of colorectal neoplasia. Carcinogenesis 34: 2512-2520.
7. Wang H, Flannery SM, Dickhofer S, Huhn S, George J, et al. (2014) A coding IRAK2 protein variant compromises Toll-like receptor (TLR) signaling and is associated with colorectal cancer survival. J Biol Chem 289: 23123-23131.
8. Kidd LC, Rogers EN, Yeyeodu ST, Jones DZ, Kimbro KS (2013) Contribution of toll-like receptor signaling pathways to breast tumorigenesis and treatment. Breast Cancer (Dove Med Press) 5: 43-51.
9. Xu H, Wu Q, Dang S, Jin M, Xu J, et al. (2011) Alteration of CXCR7 expression mediated by TLR4 promotes tumor cell proliferation and migration in human colorectal carcinoma. PLoS One 6: e27399.
10. Gunter MJ, Stolzenberg-Solomon R, Cross AJ, Leitzmann MF, Weinstein S, et al. (2006) A prospective study of serum C-reactive protein and colorectal cancer risk in men. Cancer Res 66: 2483-2487.
11. Lashner BA, Turner BC, Bostwick DG, Frank PH, Hanauer SB (1990) Dysplasia and cancer complicating strictures in ulcerative colitis. Dig Dis Sci 35: 349-352.
12. Fumery M, Pineton de Chambrun G, Stefanescu C, Buisson A, Bressenot A, et al. (2015) Detection of dysplasia or cancer in 3.5% of patients with inflammatory bowel disease and colonic strictures. Clin Gastroenterol Hepatol 13: 1770-1775.
13. Sonnenberg A, Genta RM (2015) Epithelial dysplasia and cancer in IBD strictures. J Crohns Colitis 9: 769-775.
14. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF (2017) Ulcerative colitis. Lancet 389: 1756-1770.
15. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, et al. (2013) Inflammation-induced cancer: Crosstalk between tumours, immune cells and microorganisms. Nat Rev Cancer 13: 759-771.
16. Hussain SP, Amstad P, Raja K, Ambs S, Nagashima M, et al. (2000) Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: A cancer-prone chronic inflammatory disease. Cancer Res 60: 3333-3337.
17. Hofseth LJ, Wargovich MJ (2007) Inflammation, cancer, and targets of ginseng. J Nutr 137: 183S-185S.
18. Pradere JP, Dapito DH, Schwabe RF (2014) The Yin and Yang of toll-like receptors in cancer. Oncogene 33: 3485-3495.
19. Rakoff-Nahoum S, Medzhitov R (2009) Toll-like receptors and cancer. Nat Rev Cancer 9: 57-63.

20. Yu L, Wang L, Chen S (2013) Dual character of Toll-like receptor signaling: Pro-tumorigenic effects and anti-tumor functions. Biochim Biophys Acta 1835: 144-154.

21. Hagerling C, Casbon AJ, Werb Z (2015) Balancing the innate immune system in tumor development. Trends Cell Biol 25: 214-220.

22. West NR, McCuaig S, Franchini F, Powrie F (2015) Emerging cytokine networks in colorectal cancer. Nat Rev Immunol 15: 615-629.

23. Calon A, Espinet E, Palomo-Ponce S, Tauriello DV, Iglesias M, et al. (2012) Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. Cancer Cell 22: 571-584.

24. Neuzillet C, Tijeras-Raballand A, Cohen R, Cros J, Faivre S, et al. (2015) Targeting the TGFbeta pathway for cancer therapy. Pharmacol Ther 147: 22-31.

25. Chong CR, Janne PA (2013) The quest to overcome resistance to EGFR-targeted therapies in cancer. Nat Med 19: 1389-1400.

26. Martins SF, Garcia EA, Luz MA, Pardal F, Rodrigues M, et al. (2013) Clinicopathological correlation and prognostic significance of VEGF-A, VEGF-C, VEGFR-2 and VEGFR-3 expression in colorectal cancer. Cancer Genomics Proteomics 10: 55-67.

27. Frosali S, Pagliari D, Gambassi G, Landolfi R, Pandolfi F, et al. (2015) How the intricate interaction among toll-like receptors, microbiota, and intestinal immunity can influence gastrointestinal pathology. J Immunol Res 2015: 489821.

28. Allali I, Delgado S, Marron PI, Astudillo A, Yeh JJ, et al. (2015) Gut microbiome compositional and functional differences between tumor and non-tumor adjacent tissues from cohorts from the US and Spain. Gut Microbes 6: 161-172.

29. Leung A, Tsoi H, Yu J (2015) *Fusobacterium* and *Escherichia*: Models of colorectal cancer driven by microbiota and the utility of microbiota in colorectal cancer screening. Expert Rev Gastroenterol Hepatol 9: 651-657.

30. Lowe EL, Crother TR, Rabizadeh S, Hu B, Wang H, et al. (2010) Toll-like receptor 2 signaling protects mice from tumor development in a mouse model of colitis-induced cancer. PLoS One 5: e13027.

31. Nihon-Yanagi Y, Terai K, Murano T, Matsumoto T, Okazumi S (2012) Tissue expression of Toll-like receptors 2 and 4 in sporadic human colorectal cancer. Cancer Immunol Immunother 61: 71-77.

32. Salcedo R, Worschech A, Cardone M, Jones Y, Gyulai Z, et al. (2010) MyD88-mediated signaling prevents development of adenocarcinomas of the colon: Role of interleukin 18. J Exp Med 207: 1625-1636.

33. Grasa L, Abecia L, Forcen R, Castro M, de Jalon JA, et al. (2015) Antibiotic-induced depletion of murine microbiota induces mild inflammation and changes in toll-like receptor patterns and intestinal motility. Microb Ecol 70: 835-848.

34. Lu CC, Kuo HC, Wang FS, Jou MH, Lee KC, et al. (2014) Upregulation of TLRs and IL-6 as a marker in human colorectal cancer. Int J Mol Sci 16: 159-177.

35. Tchorzewski M, Lewkowicz P, Dziki A, Tchorzewski H (2014) Expression of toll-like receptors on human rectal adenocarcinoma cells. Arch Immunol Ther Exp (Warsz) 62: 247-251.

36. Semlali A, Reddy Parine N, Arafah M, Mansour L, Azzi A, et al. (2016) Expression and polymorphism of toll-like receptor 4 and effect on NF-kappaB mediated inflammation in colon cancer patients. PLoS One 11: e0146333.

37. Huang HY, Zhang ZJ, Cao CB, Wang N, Liu FF, et al. (2014) The TLR4/NF-kappaB signaling pathway mediates the growth of colon cancer. Eur Rev Med Pharmacol Sci 18: 3834-3843.

38. Santaolalla R, Sussman DA, Ruiz JR, Davies JM, Pastorini C, et al. (2013) TLR4 activates the beta-catenin pathway to cause intestinal neoplasia. PLoS One 8: e63298.

39. Doan HQ, Bowen KA, Jackson LA, Evers BM (2009) Toll-like receptor 4 activation increases Akt phosphorylation in colon cancer cells. Anticancer Res 29: 2473-2478.

40. Wang EL, Qian ZR, Nakasono M, Tanahashi T, Yoshimoto K, et al. (2010) High expression of Toll-like receptor 4/myeloid differentiation factor 88 signals correlates with poor prognosis in colorectal cancer. Br J Cancer 102: 908-915.

41. Sussman DA, Santaolalla R, Bejarano PA, Garcia-Buitrago MT, Perez MT, et al. (2014) *In silico* and *Ex vivo* approaches identify a role for toll-like receptor 4 in colorectal cancer. J Exp Clin Cancer Res 33: 45.

42. Eiro N, Gonzalez L, Gonzalez LO, Fernandez-Garcia B, Andicoechea A, et al. (2013) Toll-like receptor-4 expression by stromal fibroblasts is associated with poor prognosis in colorectal cancer. J Immunother 36: 342-349.

43. Hsu RY, Chan CH, Spicer JD, Rousseau MC, Giannias B, et al. (2011) LPS-induced TLR4 signaling in human colorectal cancer cells increases beta1 integrin-mediated cell adhesion and liver metastasis. Cancer Res 71: 1989-1998.

44. Jorissen RN, Gibbs P, Christie M, Prakash S, Lipton L, et al. (2009) Metastasis-associated gene expression changes predict poor outcomes in patients with Dukes stage B and C colorectal cancer. Clin Cancer Res 15: 7642-7651.

45. Lips EH, van Eijk R, de Graaf EJ, Oosting J, De Miranda NF, et al. (2008) Integrating chromosomal aberrations and gene expression profiles to dissect rectal tumorigenesis. BMC Cancer 8: 314.

46. Thorsteinsson M, Kirkeby LT, Hansen R, Lund LR, Sorensen LT, et al. (2012) Gene expression profiles in stages II and III colon cancers: application of a 128-gene signature. Int J Colorectal Dis 27: 1579-1586.

47. Smith JJ, Deane NG, Wu F, Merchant NB, Zhang B, et al. (2010) Experimentally derived metastasis gene expression profile predicts recurrence and death in patients with colon cancer. Gastroenterology 138: 958-968.

48. Staub E, Groene J, Heinze M, Mennerich D, Roepcke S, et al. (2009) An expression module of WIPF1-coexpressed genes identifies patients with favorable prognosis in three tumor types. J Mol Med (Berl) 87: 633-644.

49. De Sousa EMF, Colak S, Buikhuisen J, Koster J, Cameron K, et al. (2011) Methylation of cancer-stem-cell-associated Wnt target genes predicts poor prognosis in colorectal cancer patients. Cell Stem Cell 9: 476-485.

50. Reid JF, Gariboldi M, Sokolova V, Capobianco P, Lampis A, et al. (2009) Integrative approach for prioritizing cancer genes in sporadic colon cancer. Genes Chromosomes Cancer 48: 953-962.

51. Kaiser S, Park YK, Franklin JL, Halberg RB, Yu M, et al. (2007) Transcriptional recapitulation and subversion of embryonic colon development by mouse colon tumor models and human colon cancer. Genome Biol 8: R131.

52. Zhu G, Huang Q, Huang Y, Zheng W, Hua J, et al. (2016) Lipopolysaccharide increases the release of VEGF-C that enhances cell motility and promotes lymphangiogenesis and lymphatic metastasis through the TLR4- NF-kappaB/JNK pathways in colorectal cancer. Oncotarget 7: 73711-73724.

53. Tang XY, Zhu YQ, Wei B, Wang H (2010) Expression and functional research of TLR4 in human colon carcinoma. Am J Med Sci 339: 319-326.

54. Kantola T, Klintrup K, Vayrynen JP, Vornanen J, Bloigu R, et al. (2012) Stage-dependent alterations of the serum cytokine pattern in colorectal carcinoma. Br J Cancer 107: 1729-1736.

55. Tye H, Jenkins BJ (2013) Tying the knot between cytokine and toll-like receptor signaling in gastrointestinal tract cancers. Cancer Sci 104: 1139-1145.

56. Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, et al. (2000) Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. J Immunol 164: 966-972.

57. Tsuchiya T, Tsuno NH, Asakage M, Yamada J, Yoneyama S, et al. (2008) Apoptosis induction by p38 MAPK inhibitor in human colon cancer cells. Hepatogastroenterology 55: 930-935.

58. Laird MH, Rhee SH, Perkins DJ, Medvedev AE, Piao W, et al. (2009) TLR4/MyD88/PI3K interactions regulate TLR4 signaling. J Leukoc Biol 85: 966-977.

59. O'Leary DP, Bhatt L, Woolley JF, Gough DR, Wang JH, et al. (2012) TLR-4 signalling accelerates colon cancer cell adhesion via NF-kappaB mediated transcriptional up-regulation of Nox-1. PLoS One 7: e44176.

60. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, et al. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410: 1099-1103.

61. Uematsu S, Fujimoto K, Jang MH, Yang BG, Jung YJ, et al. (2008) Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat Immunol 9: 769-776.

62. Uematsu S, Jang MH, Chevrier N, Guo Z, Kumagai Y, et al. (2006) Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. Nat Immunol 7: 868-874.

63. Vijay-Kumar M, Aitken JD, Kumar A, Neish AS, Uematsu S, et al. (2008) Toll-like receptor 5-deficient mice have dysregulated intestinal gene expression

and nonspecific resistance to Salmonella-induced typhoid-like disease. Infect Immun 76: 1276-1281.

64. Carvalho FA, Koren O, Goodrich JK, Johansson ME, Nalbantoglu I, et al. (2012) Transient inability to manage proteobacteria promotes chronic gut inflammation in TLR5-deficient mice. Cell Host Microbe 12: 139-152.

65. Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, et al. (2004) Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. Gastroenterology 127: 80-93.

66. Swidsinski A, Khilkin M, Kerjaschki D, Schreiber S, Ortner M, et al. (1998) Association between intraepithelial Escherichia coli and colorectal cancer. Gastroenterology 115: 281-286.

67. Rhee SH, Im E, Pothoulakis C (2008) Toll-like receptor 5 engagement modulates tumor development and growth in a mouse xenograft model of human colon cancer. Gastroenterology 135: 518-528.

68. Nishimura M, Naito S (2005) Tissue-specific mRNA expression profiles of human toll-like receptors and related genes. Biol Pharm Bull 28: 886-892.

69. Lee BL, Barton GM (2014) Trafficking of endosomal Toll-like receptors. Trends Cell Biol 24: 360-369.

70. Pohar J, Pirher N, Bencina M, Mancek-Keber M, Jerala R (2014) The ectodomain of TLR3 receptor is required for its plasma membrane translocation. PLoS One 9: e92391.

71. Pohar J, Pirher N, Bencina M, Mancek-Keber M, Jerala R (2013) The role of UNC93B1 protein in surface localization of TLR3 receptor and in cell priming to nucleic acid agonists. J Biol Chem 288: 442-454.

72. Xagorari A, Chlichlia K (2008) Toll-like receptors and viruses: Induction of innate antiviral immune responses. Open Microbiol J 2: 49-59.

73. Miettinen M, Sareneva T, Julkunen I, Matikainen S (2001) IFNs activate toll-like receptor gene expression in viral infections. Genes Immun 2: 349-355.

74. Nojiri K, Sugimoto K, Shiraki K, Tameda M, Inagaki Y, et al. (2013) The expression and function of Toll-like receptors 3 and 9 in human colon carcinoma. Oncol Rep 29: 1737-1743.

75. Xiang L, Wang S, Jin X, Duan W, Ding X, et al. (2012) Expression of BMP2, TLR3, TLR4 and COX2 in colorectal polyps, adenoma and adenocarcinoma. Mol Med Rep 6: 973-976.

76. Niedzielska I, Niedzielski Z, Tkacz M, Orawczyk T, Ziaja K, et al. (2009) Toll-like receptors and the tendency of normal mucous membrane to transform to polyp or colorectal cancer. J Physiol Pharmacol 60: 65-71.

77. Grimm M, Kim M, Rosenwald A, Heemann U, Germer CT, et al. (2010) Toll-like receptor (TLR) 7 and TLR8 expression on CD133+ cells in colorectal cancer points to a specific role for inflammation-induced TLRs in tumourigenesis and tumour progression. Eur J Cancer 46: 2849-2857.

78. Thada S, Valluri VL, Gaddam SL (2013) Influence of Toll-like receptor gene polymorphisms to tuberculosis susceptibility in humans. Scand J Immunol 78: 221-229.

79. Kaczanowska S, Joseph AM, Davila E (2013) TLR agonists: Our best frenemy in cancer immunotherapy. J Leukoc Biol 93: 847-863.

80. Rahman AH, Taylor DK, Turka LA (2009) The contribution of direct TLR signaling to T cell responses. Immunol Res 45: 25-36.

81. Furi I, Sipos F, Germann TM, Kalmar A, Tulassay Z, et al. (2013) Epithelial toll-like receptor 9 signaling in colorectal inflammation and cancer: Clinico-pathogenic aspects. World J Gastroenterol 19: 4119-4126.

82. Gribar SC, Sodhi CP, Richardson WM, Anand RJ, Gittes GK, et al. (2009) Reciprocal expression and signaling of TLR4 and TLR9 in the pathogenesis and treatment of necrotizing enterocolitis. J Immunol 182: 636-646.

83. Coste I, Le Corf K, Kfoury A, Hmitou I, Druillennec S, et al. (2010) Dual function of MyD88 in RAS signaling and inflammation, leading to mouse and human cell transformation. J Clin Invest 120: 3663-3667.

84. Rakoff-Nahoum S, Medzhitov R (2007) Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science 317: 124-127.

85. Cataisson C, Salcedo R, Hakim S, Moffitt BA, Wright L, et al. (2012) IL-1R-MyD88 signaling in keratinocyte transformation and carcinogenesis. J Exp Med 209: 1689-1702.

86. Kfoury A, Le Corf K, El Sabeh R, Journeaux A, Badran B, et al. (2013) MyD88 in DNA repair and cancer cell resistance to genotoxic drugs. J Natl Cancer Inst 105: 937-946.

87. Sijbers AM, Van der Spek PJ, Odijk H, Van den Berg J, Van Duin M, et al. (1996) Mutational analysis of the human nucleotide excision repair gene ERCC1. Nucleic Acids Res 24: 3370-3380.

88. Proenca MA, De Oliveira JG, Cadamuro AC, Succi M, Netinho JG, et al. (2015) TLR2 and TLR4 polymorphisms influence mRNA and protein expression in colorectal cancer. World J Gastroenterol 21: 7730-7741.

89. Noguchi E, Nishimura F, Fukai H, Kim J, Ichikawa K, et al. (2004) An association study of asthma and total serum immunoglobin E levels for Toll-like receptor polymorphisms in a Japanese population. Clin Exp Allergy 34: 177-183.

90. Pimentel-Nunes P, Teixeira AL, Pereira C, Gomes M, Brandao C, et al. (2013) Functional polymorphisms of Toll-like receptors 2 and 4 alter the risk for colorectal carcinoma in Europeans. Dig Liver Dis 45: 63-69.

91. Sauna ZE, Kimchi-Sarfaty C, Ambudkar SV, Gottesman MM (2007) The sounds of silence: synonymous mutations affect function. Pharmacogenomics 8: 527-532.

92. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, et al. (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315: 525-528.

93. Slattery ML, Herrick JS, Bondurant KL, Wolff RK (2012) Toll-like receptor genes and their association with colon and rectal cancer development and prognosis. Int J Cancer 130: 2974-2980.

94. Boada-Romero E, Letek M, Fleischer A, Pallauf K, Ramon-Barros C, et al. (2013) TMEM59 defines a novel ATG16L1-binding motif that promotes local activation of LC3. EMBO J 32: 566-582.

95. Omrane I, Benammar-Elgaaied A (2015) The immune microenvironment of the colorectal tumor: Involvement of immunity genes and microRNAs belonging to the TH17 pathway. Biochim Biophys Acta 1856: 28-38.

96. Eyking A, Ey B, Runzi M, Roig AI, Reis H, et al. (2011) Toll-like receptor 4 variant D299G induces features of neoplastic progression in Caco-2 intestinal cells and is associated with advanced human colon cancer. Gastroenterology 141: 2154-2165.

97. Boraska Jelavic T, Barisic M, Drmic Hofman I, Boraska V, Vrdoljak E, et al. (2006) Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. Clin Genet 70: 156-160.

98. Kutikhin AG, Yuzhalin AE, Volkov AN, Zhivotovskiy AS, Brusina EB (2014) Correlation between genetic polymorphisms within IL-1B and TLR4 genes and cancer risk in a Russian population: A case-control study. Tumour Biol 35: 4821-4830.

99. Davoodi H, Seow HF (2011) Variant Toll-like receptor4 (Asp299Gly and Thr399Ile alleles) and Toll-like receptor2 (Arg753Gln and Arg677Trp alleles) in colorectal cancer. Iran J Allergy Asthma Immunol 10: 91-99.

100. Sheng WY, Yong Z, Yun Z, Hong H, Hai LL (2015) Toll-like receptor 4 gene polymorphisms and susceptibility to colorectal cancer: A meta-analysis and review. Arch Med Sci 11: 699-707.

101. Li XX, Sun GP, Meng J, Li X, Tang YX, et al. (2014) Role of toll-like receptor 4 in colorectal carcinogenesis: A meta-analysis. PLoS One 9: e93904.

102. Kopp TI, Andersen V, Tjonneland A, Vogel U (2015) Polymorphisms in NFKB1 and TLR4 and interaction with dietary and lifestyle factors in relation to colorectal cancer in a Danish prospective case-cohort study. PLoS One 10: e0116394.

103. Tsilidis KK, Helzlsouer KJ, Smith MW, Grinberg V, Hoffman-Bolton J, et al. (2009) Association of common polymorphisms in IL10, and in other genes related to inflammatory response and obesity with colorectal cancer. Cancer Causes Control 20: 1739-1751.

104. Landi S, Gemignani F, Bottari F, Gioia-Patricola L, Guino E, et al. (2006) Polymorphisms within inflammatory genes and colorectal cancer. J Negat Results Biomed 5: 15.

105. Guo Q, Zhu J, Xia B (2006) Polymorphism of CD14 gene but not the mutation of TLR4 gene is associated with colorectal cancer in Chinese patients. J Gastroenterol Hepatol 21: 92-97.

106. Ge J, Zhu L, Zhou J, Li G, Li Y, et al. (2015) Association between co-inhibitory molecule gene tagging single nucleotide polymorphisms and the risk of colorectal cancer in Chinese. J Cancer Res Clin Oncol 141: 1533-1544.

107. Formica V, Cereda V, Di Bari MG, Grenga I, Tesauro M, et al. (2013) Peripheral CD45RO, PD-1, and TLR4 expression in metastatic colorectal cancer patients treated with bevacizumab, fluorouracil, and irinotecan (FOLFIRI-B). Med Oncol 30: 743.

108. Stier S, Maletzki C, Klier U, Linnebacher M (2013) Combinations of TLR ligands: A promising approach in cancer immunotherapy. Clin Dev Immunol 2013: 271246.

109. Pham TN, Hong CY, Min JJ, Rhee JH, Nguyen TA, et al. (2010) Enhancement of antitumor effect using dendritic cells activated with natural killer cells in the presence of Toll-like receptor agonist. Exp Mol Med 42: 407-419.

110. Gosu V, Basith S, Kwon OP, Choi S (2012) Therapeutic applications of nucleic acids and their analogues in Toll-like receptor signaling. Molecules 17: 13503-13529.

111. Sharma S, Zhu L, Davoodi M, Harris-White M, Lee JM, et al. (2013) TLR3 agonists and proinflammatory antitumor activities. Expert Opin Ther Targets 17: 481-483.

112. Seya T, Azuma M, Matsumoto M (2013) Targeting TLR3 with no RIG-I/MDA5 activation is effective in immunotherapy for cancer. Expert Opin Ther Targets 17: 533-544.

113. Matsumoto M, Tatematsu M, Nishikawa F, Azuma M, Ishii N, et al. (2015) Defined TLR3-specific adjuvant that induces NK and CTL activation without significant cytokine production *in vivo*. Nat Commun 6: 6280.

114. Wang D, Precopio M, Lan T, Yu D, Tang JX, et al. (2010) Antitumor activity and immune response induction of a dual agonist of Toll-like receptors 7 and 8. Mol Cancer Ther 9: 1788-1797.

115. Taura M, Fukuda R, Suico MA, Eguma A, Koga T, et al. (2010) TLR3 induction by anticancer drugs potentiates poly I:C-induced tumor cell apoptosis. Cancer Sci 101: 1610-1617.

116. de Angelis PM, Fjell B, Kravik KL, Haug T, Tunheim SH, et al. (2004) Molecular characterizations of derivatives of HCT116 colorectal cancer cells that are resistant to the chemotherapeutic agent 5-fluorouracil. Int J Oncol 24: 1279-1288.

117. Kojouharov BM, Brackett CM, Veith JM, Johnson CP, Gitlin, II, et al. (2014) Toll-like receptor-5 agonist Entolimod broadens the therapeutic window of 5-fluorouracil by reducing its toxicity to normal tissues in mice. Oncotarget 5: 802-814.

118. Adlard AL, Dovedi SJ, Telfer BA, Koga-Yamakawa E, Pollard C, et al. (2014) A novel systemically administered Toll-like receptor 7 agonist potentiates the effect of ionizing radiation in murine solid tumor models. Int J Cancer 135: 820-829.

119. Ito H, Ando T, Arioka Y, Saito K, Seishima M (2015) Inhibition of indoleamine 2,3-dioxygenase activity enhances the anti-tumour effects of a Toll-like receptor 7 agonist in an established cancer model. Immunology 144: 621-630.

120. Scholch S, Rauber C, Tietz A, Rahbari NN, Bork U, et al. (2015) Radiotherapy combined with TLR7/8 activation induces strong immune responses against gastrointestinal tumors. Oncotarget 6: 4663-4676.

121. Northfelt DW, Ramanathan RK, Cohen PA, Von Hoff DD, Weiss GJ, et al. (2014) A phase I dose-finding study of the novel Toll-like receptor 8 agonist VTX-2337 in adult subjects with advanced solid tumors or lymphoma. Clin Cancer Res 20: 3683-3691.

122. Kim IY, Yan X, Tohme S, Ahmed A, Cordon-Cardo C, et al. (2012) CpG ODN, Toll like receptor (TLR)-9 agonist, inhibits metastatic colon adenocarcinoma in a murine hepatic tumor model. J Surg Res 174: 284-290.

123. Schmoll HJ, Wittig B, Arnold D, Riera-Knorrenschild J, Nitsche D, et al. (2014) Maintenance treatment with the immunomodulator MGN1703, a Toll-like receptor 9 (TLR9) agonist, in patients with metastatic colorectal carcinoma and disease control after chemotherapy: A randomised, double-blind, placebo-controlled trial. J Cancer Res Clin Oncol 140: 1615-1624.

124. Rosa R, Melisi D, Damiano V, Bianco R, Garofalo S, et al. (2011) Toll-like receptor 9 agonist IMO cooperates with cetuximab in K-ras mutant colorectal and pancreatic cancers. Clin Cancer Res 17: 6531-6541.

125. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, et al. (2007) Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 13: 1050-1059.

126. Maitra R, Augustine T, Dayan Y, Chandy C, Coffey M, et al. (2017) Toll like receptor 3 as an immunotherapeutic target for KRAS mutated colorectal cancer. Oncotarget 8: 35138-35153.

127. Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, et al. (2013) MUC1 vaccine for individuals with advanced adenoma of the colon: A cancer immunoprevention feasibility study. Cancer Prev Res 6: 18-26.

128. Li TT, Ogino S, Qian ZR (2014) Toll-like receptor signaling in colorectal cancer: Carcinogenesis to cancer therapy. World J Gastroenterol 20: 17699-17708.

129. Chan E, Kwak EL, Hwang J, Heiskala M, De La Bourdonnaye G, et al. (2015) Open-label phase 1b study of FOLFIRI plus cetuximab plus IMO-2055 in patients with colorectal cancer who have progressed following chemotherapy for advanced or metastatic disease. Cancer Chemother Pharmacol 75: 701-709.