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Effect Of Vitamin D In Metastasis Of Colorectal Cancer: A Study Revealing The Pathophysiological Illustration Of Bio-Molecular Interactions

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Abstract

Globally, ~1,800,000 new cases are diagnosed every year and now accounts for approximately 10% of cancer-related mortality in western countries and the number of new cases may increase to nearly 2.5 million in 2035. In the pathobiology of CRC included various different factors and bio-molecules aggravation such as a immunohistochemistry indicated increased TMPRSS13 protein levels,Chemokine ligand 20 (CCL20) mediated abnormally expression of Engrailed-2 (EN2),persistent inflammation, hypercoagulation and IL-6/JAK2/STAT3 signaling, which activated PI3K/AKT signaling,Notchsignalling, Hedgehog pathway,TGF-β (transforming growth factor-β)/SMAD,and RAS/rapidly accelerated fibrosarcoma (RAF).However, great progress has been achieved in multimodality therapy of CRC, the prognosis of late-stage CRC is still unsatisfactory due to distant metastasis and relapse. Although, targeted therapies have been successful in the treatment of some types of cancers, such as breast cancer, they have limited efficacy in adjuvant treatment of colorectal cancer (i.e., cetuximab, panitumumab, bevacizumab, ramucirumab, zivaflibercept, and regorafenib) and add relatively small survival benefits for those with advanced disease. From previous years Wnt/β-catenin canonical signalling pathway coming into picture of CRC.According to the Cancer Genome Atlas more than 94% of cases of colorectal cancer have mutations in one or more Wnt/β-catenin signalling pathway components, suggesting that Wnt/β-catenin canonical signallingmechanism are important hallmark of both the early stages and the late stages for cell proliferation and migration and tumorigenesis in CRC. Thus, our primary aim in study to investigate different target sites of Wnt/β-cateninsignalling and up regulatory axis that involve in progression of CRC. And secondary aim to identify the role of vitamin D in influencing site of the targeted Wnt/β-catenin pathway.

Keywords: Colorectal cancer, Renin angiotensinsystem,Wnt/*β*-catenin canonical pathway, Cyclooxygenase-2, Pro-Renin receptors, Vit. D

1. Introduction

According to the American Cancer Society, colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths in both genders in the United States(1). Globally, ~1,800,000 new cases are diagnosed every year and now accounts for approximately 10% of cancer-related mortality in western countries and the number of new cases may increase to nearly 2.5 million in 2035.According to the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute, ~21% of CRC patients have metastatic disease with a 5-year survival rate of only 14%.In addition, with the change in lifestyle, including high-fat diets (HFDs), tobacco use, less or lack of exercise and obesity, the incidence of CRC has increased rapidly in developing countries(2-6). However, great progress has been achieved in multimodality therapy of CRC, the prognosis of late-stage CRC is still unsatisfactory due to distant metastasis and relapse(7, 8). Early diagnosis of disease can lead to successful treatment through surgical interventions, although the prognosis for advanced and metastatic CRC is poor due to limited medical treatment options. Fluorouracil (5-FU) and its pro-drug form capecitabine are currently the most frequently used agents, alone or in combination with drugs such as oxaliplatin and irinotecan(9-11).Furthermore, targeted therapies have been successful in the treatment of some types of cancers, such as breast cancer, they have limited efficacy in adjuvant treatment of colorectal cancer (i.e., cetuximab, panitumumab, bevacizumab,ramucirumab, zivaflibercept, and regorafenib) and add relatively small survival benefits for those with advanced disease(12-14). Therefore, there is an urgent need to develop novel drug regimens for patients suffering from advanced CRC. To this end, understanding the molecular mechanisms driving CRC represents a critical step toward the development of novel targeted therapeutics for this particularly deadly type of cancer.

2. Selection of literature for review

The potentially relevant studies were retrieved from the ScienceDirect/Medline/PubMed/Public library of science/Mendeley/Springer link and Google Scholar. Multiple keywords were used for the literature search both alone as well as in combination. Some of the important keywords used for literature search were ‘Epidemiology of colon cancer’, ‘Therapeutic strategy for treatment of coloncancer’,‘Wnt beta-catenin pathway mediated colorectal cancer’, ‘Involvement of PRR in wnt beta-catenin pathway’, ‘Pathogenesis of colorectal cancer’, ‘Vit. D mediated action for treatment in colorectal metastasis’, ‘effect of vit. D on Wnt canonical pathway’ ‘effect of vit. D on RAS system’ or ‘Role of Cox-2 in colorectal cancer’, in combination with PRR activity. Only articles with English language were considered in the present study. The reference lists of retrieved articles were also screened to find relevant articles that were not identified by the initial search strategy.

3. Involvement of bio-molecular interactions in pathogenesis of colorectal cancer

Several studies have been associated with the different factors and bio-molecules aggravation in CRC such as a immunohistochemistry indicated increased TMPRSS13 protein levels(15), Chemokine ligand 20 (CCL20) mediated abnormally expression of Engrailed-2 (EN2)(16),aberrantly upregulatedWnt/β-catenin

canonical signaling(17), persistent inflammation, hypercoagulation(18) and IL-6/JAK2/STAT3 pathway, which activated PI3K/AKT signaling(19), Notch(20), Hedgehog(21), and TGF-β (transforming growth factor-β)/SMAD(22, 23), RAS/rapidly accelerated fibrosarcoma (RAF)(24), contain ideal targeted sites in CRC. However, according to the Cancer Genome Atlas more than 94% of cases of colorectal cancer have mutations in one or more Wnt/β-catenin signaling pathway components(25),suggesting that Wnt/β-catenin canonical signaling mechanism are important hallmark of both the early stages and the late stages for cell proliferation and migration and tumorigenesis in CRC (Figure 1).

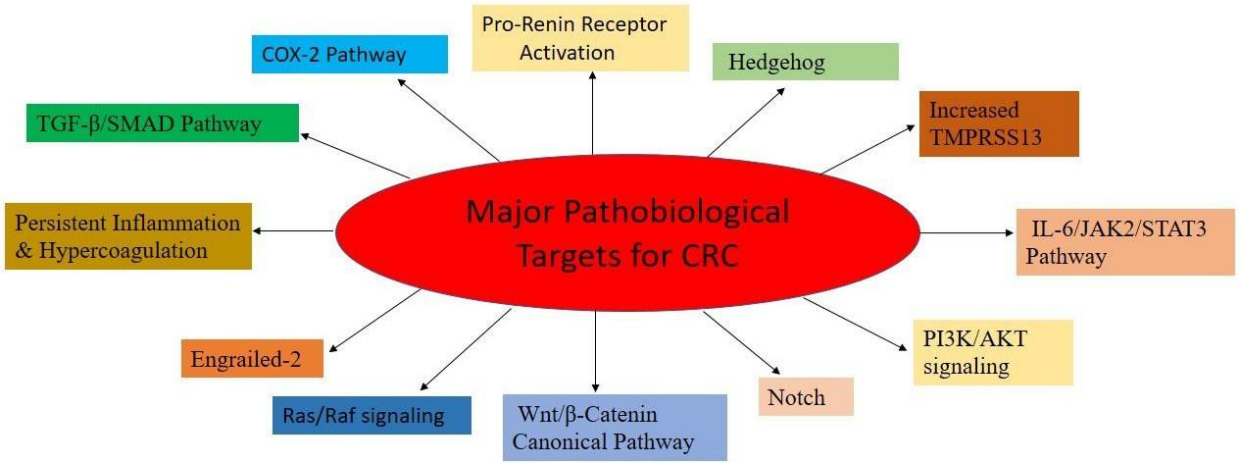

Figure 1: Express major pathobiological targets for drug therapy in CRC

4. Cyclooxygenase-2 mediated response

Later work on the biology of COX enzymes led to the distinction between two COX enzymes: COX-1(26) and COX-2(27). A third form (COX-3) has also been reported(28), although recent studies indicate that this represents a splice variant of COX-1 that encodes a truncated protein lacking enzymatic activity(29). In humans, COX-1 is found constitutively expressed in a wide range of tissues including the kidney, lung, stomach, small intestine and colon. Thus, COX-1 is considered a housekeeping enzyme responsible for maintaining basal prostaglandin levels important for tissue homeostasis. In contrast, most tissues do not normally express COX-2 constitutively, notable exceptions including the central nervous system(30), kidneys(31) and seminal vesicles(32). However, the stimulation of COX-2 expression in Src-transformed fibroblasts(27), endothelial cells and monocytes treated with the tumour promoter tetradecanoyl-phorbol-acetate(33) or lipopolysaccharide(34) led to the notion that COX-2 is an inducible enzyme that produces prostaglandins during inflammatory and tumorigenic settings. Because of this, there has been a fervent interest in studying the biology of COX-2 in relation to tumorigenesis, particularly with regard to colorectal tumorigenesis where its actions appear to have a major impact on tumour development. COX-2 is the inducible form of cyclooxygenase enzymes and guilty of the production of prostaglandins, mainly PGE_2, involved in inflammation and tumor framework. Transcription of COX-2 gene is controlled by several consensus sequences in the promoter region, and a TCF-binding elements

have been identified as functional Wnt/β-Catenin responsive elements within the human COX-2 promoter in both colorectal and gastric cancer cell lines(35, 36). On the other hand, COX-2/PGE_2 pathway can inactivate the GSK3 β-mediated phosphorilation of β-Catenin and then trigger the activation of Wnt/β-Cateninsignalling(37). An interesting model depicted the interplay between COX-2/PGE_2 and β-Catenin during CRC induction and progression. Briefly, in normoxic conditions COX-2/PGE_2 axis promotes the stimulation of β-Catenin/TCF-4 activity whereas during hypoxia, a common status occurring in the advanced stage of cancer, β-Catenin, displaced from TCF-4, interacts with HIF-1, improves its transcriptional activity and substantially increases the expression of HIF-1 targets such as VEGF(38).Furthermore, previous preclinical study demonstrates that PRR activation is mediated by cyclooxygenase-2 (COX-2) pathway in kidney cells(39). Agents such as COX-2 inhibitors were found to be helpful in CRC prevention in terms of Wnt inhibition; however, the development of other agents that might enhance chemotherapy sensitivity, yet direct CRC-control-targeted drugs with high affinity to single targets.

5. Wnt/beta catenin canonical pathway

Canonical Wnt/β-catenin signaling plays essential roles in embryonic development and maintaining gut homeostasis(40). Inactivating mutations in APC or activating mutations in β-catenin (CTNNB1) lead to signaling overactivation and subsequent intestinal hyperplasia(41). Persistent activation of Wnt signaling featured by nuclear accumulation of β-catenin is an early event of colorectal tumorigenesis(42). Moreover,Wnt/β-catenin signaling as a hypoxia-responsive regulator in colorectal-cancer cells and through various in vitro studies, we found that hypoxia-induced Wnt/β-catenin signaling increased the occurrence of cancer stem-cell -like phenotypes and the level of Id2 expression(43), which directly interact of Rb lead to the up-regulated expression of genes that drive cells from the S phase through the G1 phase. In colorectal-cancer cells Wnt-related targets, including c-Myc, cyclin D1, MMP-7, and VEGF,CD44, LGR5, ALDH, HIF1a, SOX2 are critical contributors to tumor cell proliferation, invasion, and migratory potential(44-48). Thus, activation of Wnt/β-catenin signaling and associated gene are strong inducer of metastasis of colon cancer (Figure 2).

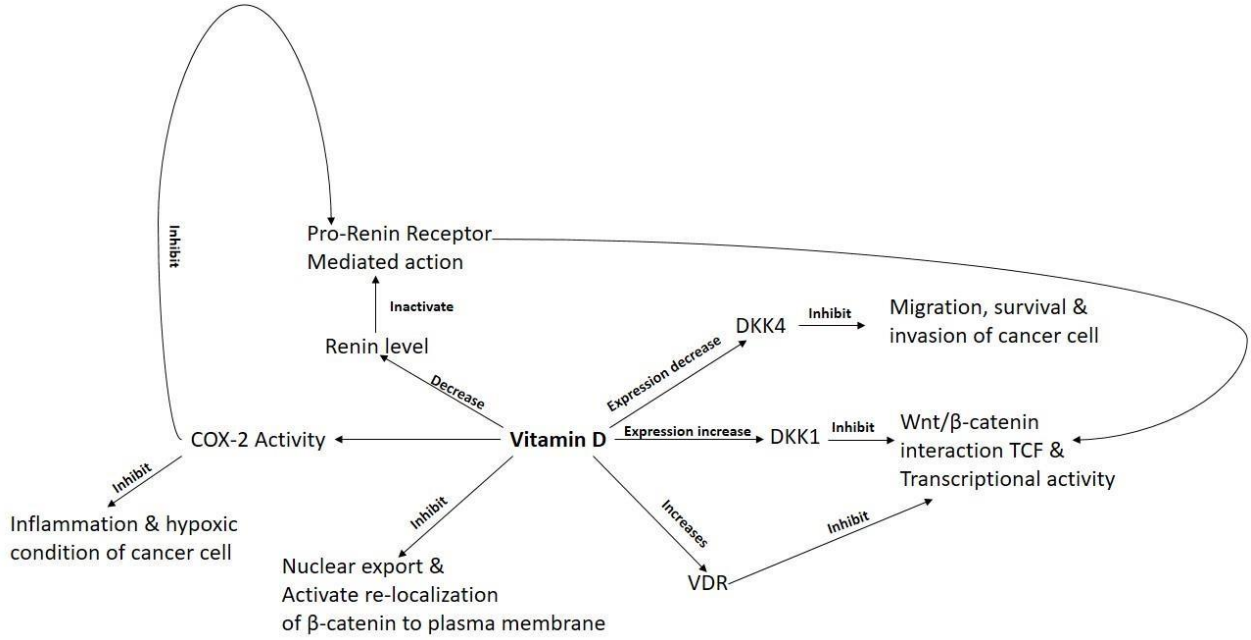

Figure 2: Illustration of Vitamin D mediated inhibition of various bio-molecules of Wnt/B-catenin in CRC

When the Wnt ligand is not bound to the receptors Frizzled and Low-density lipoprotein receptors 5/6 (LRP5/6), cytosolic β-catenin is degraded by a multiprotein complex, named destruction complex, formed by Axin, Adenomatosis polyposis coli (APC), Protein phosphatase 2A (PP2A), Glycogen synthase kinase 3 (GSK3) and Casein kinase 1α (CK1α)(49). The phosphorylation of β-catenin by CK1α and GSK3 marks it for ubiquitination and posterior degradation by the proteasomal machinery(50). The binding of Wnt ligands to its receptors triggers the signaling cascade that disassembles the β-catenin destruction complex3. Wnt signaling activation induces Disheveled (Dvl) mediated Axin membrane translocation, which binds to the cytoplasmic portion of LRP5/6(51-53). Due to the membrane position of the degradation complex, GSK3 and CK1α start to phosphorylate LRP5/6 leading to β-catenin stabilization in the cytoplasm(54). In that sense, β-catenin stabilization also occurs through Axin proteolysis catalyzed by Tankyrases (TNKs)(55). Then, β-catenin translocates to the nucleus where it will bind TCF/LEF transcription factors(56) to activate the expression of target oncogenes such as CCND1 (encodes Cyclin D1)and c-Myc(44, 57, 58). Over 80% of colorectal tumours carry loss-of-function mutation in APC and approximately 5% carry activating mutation in β-catenin(59, 60) which leads to the constitutive activation of the Wnt/β-catenin pathway and thus contributes to cancer development(41). Several studies identify Wnt/β-catenin as a master upstream regulator that controls the expression of multiple RAS genes in a synchronized fashion. The consensus TCF/LEF binding sequence is (A/T)(A/T)CAA(A/T)G. Of particular interest, bioinformatics analyses have uncovered the presence of putative TCF/LEF-binding sites in the promoter regions of all RAS genes including AGT, renin, ACE, AT1, and AT2(61). However, recent studies have shown that Wnt3 silencing remarkably attenuated the activation of the Wnt/β-catenin pathway and proliferation of CRC cells with mutation in APC or β-catenin(62).

6. Involvement of Pro-Renin receptor (PRR)withWnt mediated biological response

The (pro)renin receptor ((P)RR) was discovered and successfully cloned by Nguyen et al.(63)(Pro)renin

receptor ((P)RR), a 350-amino acid protein encoded by an X chromosome-located gene, was initially discovered as an essential component in the renin-angiotensin system (RAS) and ubiquitously expressed in the human body(64).In this review, we shall focus on the renin-angiotensin system (RAS), not RAAS, in relation to the pathophysiology of CRC. TheRAS is critical for the control of blood pressure and salt balance in mammals. The physiological maturation of prorenin into active renin takes place exclusively in the juxtaglomerular cells of the kidney(65). Renin has high substrate specificity, and its only known substrate is angiotensinogen. Renin cleaves the N terminus of circulating angiotensinogen to angiotensin I (Ang I; a decapeptide), which is then transformed in angiotensin II (Ang II; an octapeptide) by soluble or endothelial cell–associated angiotensin-converting enzyme (ACE). In the heart, the majority of Ang I is converted by chymase(66, 67). The rate-limiting step in the RAS is Ang I generation, even though the major biologically active peptide is Ang II. Ang II acts on vascular smooth muscle cells as a potent vasoconstrictor via Ang II receptors. These receptors are widely distributed and expressed by many cell types(68, 69) including colon and this peptidergic system regulates long-term biologic processes such as cell growth and proliferation(70-72). In addition, a study explored the finding of PRR is a downstream target of Wnt/β-catenin signaling and subjected to its regulation. There are putative TCF–binding sites in the promoter region of human, mouse, and rat PRR genes, raising the possibility that β-catenin can control its expression. However, Cruciat et al.(73) demonstrated that (P)RR also acts as an adaptor protein that co-locates with the Wnt receptor complex and thus contributes to the activation of Wnt/β-catenin signalling, independent of the renin-angiotensin system. Recently, accumulating evidence has revealed that the expression of (P)RR is remarkably elevated in various human cancers, such as breast carcinoma(74), pancreatic ductal adenocarcinoma(75), glioma(76), aldosterone-producing adenoma(77) and pathogenesis of CRC(78). Furthermore, a study exhibit (P)RR expression in cancers of different grades of CRCs, were classified using the WHO criteria into Grade 1, 2 and 3, which indicates well-, moderately-, and poorly-differentiated cancers, respectively. We found that (P)RR expression was generally the lowest in Grade 1 cancers, at the middle level in Grade 2 cancers, and the highest in Grade 3 cancers. In addition, (P)RR silencing also obviously reduced protein levels of active β-catenin, as well as Wnt target proteins Cyclin D1 and c-Myc. Another study explored, cyclin D1 expression was reduced following (P)RR siRNA knockdown and attenuation of Pancreatic ductal adenocarcinoma cell proliferation by (P)RR inhibition was accompanied by a substantial decrease in G0/G1 populations and an increase in the sub-G1 phase, indicating that (P)RR plays an important role in PDAC cell proliferation and inhibition of apoptosis(79). Finally, induction of (P)RR overexpression in normal human colon epithelial cells enhances the Wnt/β-catenin signalling and up-regulate LPR6 at both protein and mRNA levels and promotes cell proliferation(78). These data suggest that (P)RR is a potential biomarker for diagnosis and progression prediction, as well as a therapeutic target of CRC.

7. Potential of Vit. D foractivated biomolecule mediated response in metastasis of colorectal cancer

Vitamin D is an inexpensive, non-toxic, and easily accessible treatment that has demonstrated anti-neoplastic activities against CRC. Colon cancer cells express high levels of the vitamin D receptor (VDR)(80). In vitro studies found that by binding with VDR, vit. D can induce apoptosis of colon cancer cells and counteract aberrant WNT-β catenin signalling. Furthermore, with the same study explaining in APC(min) mice, a model of intestinal tumorigenesis, tumour burden was increased by inactivation of the VDR gene and decreased by treatment with vitamin D or its synthetic analogue (81).

Wnt reception is modulated by secreted extracellular Wnt antagonists which can be divided into two functional classes: those that bind directly to Wnts (secreted Frizzled-related proteins (SFRPs), Wnt inhibitory factor-1 (WIF-1), and Xenopus Cerberus), thereby altering their ability to bind to the Wnt receptors; and those that inhibit Wntsignalling by binding to LRP5/6 (Dickkopf (Dkk) proteins, and Wise)(82). The Dickkopf family encodes secreted proteins of 255-350 aminoacids and consists of four main members in vertebrates (Dkk-1 to -4). Dkk-1, the most widely studied member of this family, and Dkk-4 proteins act as pure inhibitors of Wnt/β-catenin signalling. In contrast, Dkk-2 and Dkk-3 can activate or inhibit the pathway depending on the cellular context(83-85). The inhibitory effect of Dkks maybe brought about by two mechanisms. First, Dkk binding toLRP5/6 can directly block the LRP-Wnt interaction(86). Andsecond, Dkks can form a ternary complex with LRP5/6 andanother class of high affinity Dkk receptors named Kremen(Krm1/2), which induces rapid endocytosis and removal ofLRP56 from the plasma membrane, thereby presumablyblocking Wnt/β-catenin signalling(87, 88).

Previous work has demonstrated that the most active vitamin D metabolite, 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3) inhibits β-catenin transcriptional activity by promoting vitamin D receptor (VDR) binding to β-catenin and the induction of Ecadherin expression. Recently, 1,25(OH)2D3 has been shown to distinctly regulate two genes encoding the extracellular Wnt inhibitors DICKKOPF-1 and DICKKOPF-4 (DKK-1, DKK-4). By an indirect transcriptional mechanism, 1,25(OH)2D3 increases the expression of DKK-1 RNA and protein, which acts as a tumour suppressor in human colon cancer cells harbouring endogenous mutations in the Wnt/β-catenin pathway. In contrast, 1,25(OH)2D3 represses DKK-4 transcription by inducing direct VDR binding to its promoter. Unexpectedly, DKK-4 is a target of the Wnt/β-catenin pathway and is up-regulated in colorectal tumours, and it has been shown to increase cell migration and invasion and to promote a proangiogenic phenotyp(89).

$1,25(OH)_2D_3$ administration suppress the expression of cyclooxygenase-2 (which catalyzes PG synthesis) and upregulation the expression of NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (which catalyzes PG degradation)resulting in overall reduction in PGs levels in prostate and breast cancer cells(90, 91). Collectively, $1,25(OH)_2D_3$ possesses strong anti-inflammatory activities in multiple cancers through different mechanisms. Since inflammation has an important role in tumour progression, using $1,25(OH)_2D_3$ as a therapeutic agent has beneficial effects on cancer prevention and treatment.Another study also shows the association. 1,25 dihydroxyvitamin D_3 downregulates the expression of renin, and deficiency leads to overexpression of renin provides a potential explanation for this association. Furthermore, defects in the vitamin D receptor signaling also promote local RAS activation in other tissues leading to detrimental effects(92). Vitamin D and its analogues inhibit cell cycle progressionregulated by cyclin-dependent kinases (CDKs) andcyclin-dependent kinase inhibitors (CKIs) (through expression of cell cycle inhibitors p21 and p27, cell adhesion molecule E-cadherin, while inhibiting transcriptional activity of β-catenin and arrest at the G0/1 to S transition) and tumor cell growth in several cancer cell lines. Such mechanisms range from preventing cell proliferation to inducing apoptosis or suppressing the expression of cell adhesion molecules and growth factors that contribute to cancer metastasis (93-97).Moreover, VDR also regulates both innate and adaptive immune responses. Althoughepithelial VDR expression alone is able to ameliorate genetic and chemical models ofcolitis, the inflammatory response can also be affected by VDR expression in bothinnate and adaptive immune cells. VDR signaling can reduce inflammation viaincreasing the secretion of anti-inflammatory cytokine and regulate the differentiationof tolerogenic dendritic cell (DC) and regulatory T-cell(98). Taken

together, these findings show that 1,25(OH)2D3 exerts a complex set of regulatory actions leading to the inhibition of the Wnt/β-catenin pathway in colon cancer cells that is in line with its protective effect against this neoplasia.

Prospective cohort studies, with circulating Calcifediol [25(OH)D] levels measured either prior to or at cancer diagnosis, consistently showed a high prevalence of vitamin D deficiency among CRC patients and those having the higher 25(OH)D levels had longer survival times than those with lower levels (99). Although observational studies support a positive association between 25(OH)D levels and survival of patients with metastatic CRC, they are not able to establish a causal role of higher 25(OH)D levels in improving survival. Vitamin D insufficiency can be a surrogate of worse health or a reflection of less favourable disease, which may confound the true relationship between vitamin D and survival. A double-blind Phase 2 randomised clinical trial, SUNSHINE,was therefore conducted to examine whether addition of high-dose vitamin D3 (4000 IU/day), versus standard-dose vitamin D3 (400 IU/day), to standard chemotherapy can improve outcomes in patients with metastatic CRC(100).

8. Conclusion

In general, we not only are encouraged by the fact that patients with CRC are living longer with plentiful choices of targeted treatments, of which one or more could ultimately be beneficial, but also expect even more individualized treatments to be developed that promote even longer survival, have fewer adverse reactions and have the potential for full recovery. The present study demonstrates that aberrant (P)RR expression promotes the carcinogenesis of CRC through the Wnt/β-catenin signalling pathway despite the presence of constitutive pathway component mutations. Therefore, (P)RR mediated activation of Wnt/β-catenin signalling is a potential biomarker for diagnosis and progression prediction, as well as a promising therapeutic target of CRC. On the other hand, vit. D, a novel treatment approaches that are safe, can delay progression, and prolong survival are urgently needed for this group of patients. Robust funding and support are therefore needed to conduct further studies on the biological mechanisms underlying the activity of vitamin D and other modifiable factors in CRC, such that their acceptance and incorporation into standard paradigms of patient care and management may be facilitated.

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