April 28, 2022

Lian-Sheng Ma

Editorial Office director and Company Editor-in-Chief

World Journal of Gastroenterology

Dear Dr. Lian-Sheng Ma:

Thank you for your letter of March 10, 2022, informing that the Invited Mini-Review (Number ID: 05774721), Manuscript NO: 74090 titled "**Tumor microenvironment involvement in colorectal cancer progression via Wnt/β-catenin pathway: providing understanding of the complex mechanism of chemoresistance**" has been found to be potentially publishable in the journal pending appropriate revision.

We have carefully reviewed the comments of the reviewers, the Science editor and the Company editor-in-chief and have modified the manuscript in response to their suggestions. We appreciate this criticism which has contributed to improving the presentation of our work.

Detailed in the enclosed sheet there is an enumeration of the changes made in our manuscript. We hope that it will be possible for you to find our paper fully acceptable for publication in the World Journal of Gastroenterology.

. Yours sincerely,

Dr. María Julia Martín

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Comments to the Editorial Office

As indicated below, we revised the manuscript according to the Editorial Office's comments and indications.

Comments to the Science Editor

We appreciate the opinion of the Science editor regarding our work. We have carefully checked the entire contribution. We have improved the language and have updated the references in the manuscript (All the added cites are listed at the end of this file)

We have attended to all the Reviewers' concerns and queries. This resulted in the restructuration of the contribution and more summarized content. We have deleted Sections 1 and 2 of the original manuscript and the summarized content of them was included in the Introduction section and in the section titled "TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC" (section 4 of the original manuscript). Also, we have now merged section 3 and section 4 of the original manuscript.

According to the information included in the MiniReview, we considered now reformulating the following section title:

"TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC"

Instead of:

"TME factors that modulate the Wnt/β-catenin pathway in CRC"

On the other hand, we have added suggested content to the remaining sections, including the role of autophagy, hypoxia, TME microbiota, extracellular vesicles, and immune evasion, among others, in the progression of CRC via the Wnt/β-catenin signaling pathway. We also better explained the aim of this work in the Introduction (last paragraph) and in the Abstract, which is to provide a detailed update of what is known about the role of the TME factors in CRC genesis, progression and chemoresistance through the modulation of the Wnt/β-catenin signaling pathway. The only published work dealing with this particular analysis dates back to 2008 (Huang D, Du X. Crosstalk between tumor cells and microenvironment via Wnt pathway in colorectal cancer dissemination. World J Gastroenterol 2008; 14: 1823. [DOI: 10.3748/wjg.14.1823]), and in this last decade, much progress has been made in understanding the pathogenesis and progression of CRC as well as the multiple modulations by the TME. We hope that after these modifications you consider our Minireview acceptable for publication.

Comments to the Company editor-in-chief

Thank you for considering that the manuscript is conditionally accepted. According to the suggestion, According to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors, we provide decomposable figures that are organized into a single PowerPoint file and the table meets the requirements of standard three-line tables, with only the top line, bottom line, and column line displayed.

We confirm that all figures are original; so, we add the copyright information on the bottom right-hand side of each picture in PowerPoint (PPT).

Comments to the reviewers:

We thank the Reviewers, the Science Editor and the Company editor-in-chief for taking the time to revise our work. We consider your opinion very valuable to improve the quality of our work. We hope that all the changes made meet your expectations. We show in this letter fragments of the revised manuscript with the modifications highlighted in red according to the suggestions of each reviewer.

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors:

This manuscript aimed at investigating the mechanism of chemoresistance of colorectal cancer. Tumor microenvironment is the "soil" for tumor breeding, the frequent interaction between tumor cells and other components in the microenvironment is considered to be the key factor leading to tumor progression and chemoresistance. Although there are some merits in this study, several issues should not be ignored.

Reviewer's comment 1.

Previous studies have demonstrated that the mechanism of T cell exhaustion in the TME is critical to improve cancer immunotherapy, and that the Wnt/β-catenin pathway has been identified as one of the most important oncogenic signaling pathways associated with immune evasion. Thus, I suggest the authors add specific content on the role of WNT signaling on the immune evasion in the TME, including effects on cells, i.e, natural killer (NK) cells, Treg cells, myeloid-derived suppressor cells (MDSC) and cytotoxic T lymphocytes (CTLs), rather than the simple content in part 6.

Reviewer's comment 2.

Immunotherapy targeting TME is an important treatment for CRC. It is a promising strategy for cancer therapy. Authors can add the effect on Wnt / β - Catenin signaling pathway regulates immune cells (such as CD8+ T cells, Tregs, DC) in TME to mediate the resistance of CRC to immunotherapy.

Author response to comments 1 and 2*:*

Dear reviewer, thank you for your valuable time reviewing our work. All your comments and suggestions have been considered so the manuscript has been modified accordingly. We have arranged and numbered your comments to facilitate the process of analyzing our revised manuscript.

For the reviewer information:

To satisfy the suggestion of all reviewers, we have deleted Sections 1 and 2 of the original manuscript and the summarized content of them was included in the Introduction section and in Section "TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC" (section 4 of the original manuscript). Also, we have now merged section 3 and section 4 of the original manuscript.

According to the information included in the MiniReview, we considered now reformulating the following section title:

"TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC"

Instead of:

"TME factors that modulate the Wnt/β-catenin pathway in CRC"

We agreed with the reviewer that the immune cells in the TME, including CD8+ T cells, play a key role in the response of CRC to therapies. We are grateful for this suggestion and we have added content on this matter, mainly when the Wnt/β-catenin is intervening since is the focus of our MiniReview. Also based on your suggestion we have added more content to section 4 (previously section 6). The corresponding changes to each section are shown below:

"**1. Dynamics established between the TME and CRC cells**

During CRC genesis, the TME and its associated signaling play a key role in tumor fate, since they facilitate the proliferation, invasion, metastasis and chemoresistance processes. Specifically, the TME is composed of extracellular matrix (ECM) proteins, mesenchymal stem cells (MSC), fibroblasts, cancer-associated fibroblasts (CAFs), endothelial cells (ECs), and tumor-infiltrating immune cells (TIICs), being this last group comprised by tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), natural killer (NK) cells, Treg cells, myeloid-derived suppressor cells (MDSC) and cytotoxic T lymphocytes (also known as CD8+ T cells), among others [5,21]."

"4. The TME and β-catenin pathway: future perspectives for CRC management

In the last decade, therapeutic inhibition of the β-catenin pathway has been considered an invaluable tool for developing new therapy regimens for patients with CRC [119–121]. Despite this, as previously stated, there are still no drugs approved for the treatment of CRC or clinical trials based on the targeting of this signaling pathway [16,121]. For these reasons, the comprehension of the regulatory mechanisms of the Wnt/β-catenin signaling pathway will grant not only expand the knowledge about the pathogenesis and evolution of CRC but also improve the treatment with the implementation of new targeted therapies. Many small molecules with β-catenin inhibitory effects have been developed such as Tankyrase inhibitors (TNKSi), R-spondin inhibitors, and Porcupine (PORCN) enzyme inhibitors. Tankyrases (TNKSs) favors Wnt signaling by inducing Axin degradation. Therefore, TNKSi including XAV939 (XAV), JW55, NVP-TNKS656 and GOO7-LK are effective approaches for inhibiting this pathway, in preclinical stage of validation [122,123]. Moon and collaborators have recently discovered that β-catenin expression influences the response of CRC-derived cells to MEK inhibitors and that TNKSi allows the resistance to this treatment to be exceeded in CRC cell lines with mutant KRAS and PI3K. These results demonstrate the possible efficacy of combined treatments with inhibitors of MEK and β-catenin signaling pathways. However, this kind of therapeutic strategy is not yet in the clinical trial phase [122], and there is even controversy on the implementation of this type of scheme [120]. Drugs that disable enzymes related to Wnt secretion are another possibility to inhibit the Wnt/ β-catenin. These compounds are denominated IWP-2, LGK974 or ETC-159 and inhibit Porcupin enzyme actions. Some R-spondin inhibitors, such as OMP-131R10

(Rosmantuzumab), are also currently under investigation for the treatment of CRC. When this ligand binds to the receptor LGR4 or LGR5, it enhances Wnt signaling, so blocking this point would consequently decrease the transcriptional activity of β-catenin [123].

One of the therapeutic targets that is being studied for CRC treatment is the use of antibodies or small molecules for the inhibition of Wnt ligands, like OMP-54F28, OMP-18R5 and OTSA101 [120]; the disruption of the transcriptional activity of β-catenin using small molecules that prevent the binding of this protein with the nuclear transcription factors TCF and LEF, such as LF3 and 2,4-diamino -quinazoline; or pharmacological drugs that stimulate β-catenin proteosomal degradation as MSAB [123,124].

In addition to the hyper-activating mutations affecting the β-catenin pathway in CRC, as previously mentioned, cross-communication through paracrine signals between tumor cells and TME cells amplify these effects. The Wnt/β-catenin pathway is key for immune cells differentiation and functioning [124], the aberrant activation of this signaling alters the activation and downregulation of the immune response, especially related to dendritic cells (DCs) and T cells [123]. In the TME, the β-catenin pathway-dependent production of cytokines, such as IL-10, by DCs has been shown to induce immune tolerance through CD4+ and CD8+ T cells [125]. Moreover, it was reported that β-catenin signaling is activated in tumors with an inflammatory microenvironment, immune evasion and poor infiltration of CD8+ T cells [123,126,127]. In CRC, this immune response is favored by the activation of β-catenin, Myc and RAS [7,128,129]. Currently known mechanisms that mediate immune exclusion and evasion through the Wnt/β-catenin pathway are: modulating the production and release of cytokines from DCs and consequently diminishing CD8+ T cells infiltration; stimulating the production and release of soluble factors like Snail and IL-1β from tumor cells and TAMs, respectively; and increasing Treg survival which are also effective inhibitors of CD8+ T cells[124,130]. To date, there are some inhibitors related to the immune response that are approved for clinical use, one of them is Programmed death-1 (PD-1) [131]. However, although immunomodulation can be effective in the treatment of CRC, resistance often occurs. Given that several of these drugs target CD8+ T cells, one of the explanations for the failure of these therapies may be the hyper-activation of the β-catenin pathway in the TME [132] . From this point of view, the current research focuses on the importance of the

development of anticancer therapies that target the Wnt/β-catenin pathway as a checkpoint to improve the efficacy of immunotherapies, mainly by restoring T-cell infiltration [123,124,126] .

In previous sections, we have described the role of several TME-derived factors that activates the β-catenin signaling pathway. Currently, clinical research is aimed at blocking or inhibiting the effects of these molecules on CRC. In our laboratory, we have found that the selective inhibition of the HGF receptor, the RTK Met, suppresses β-catenin phosphorylation at domains that favor its nuclear translocation induced by PTHrP (Novoa Diaz et al., unpublished work). Concerning this, Rimassa and his research group explored in a clinical phase II study the effect of an oral selective Met inhibitor (Tivantinib) and found that, in combination with another drug, at least 10% of patients with CRC respond better to the therapy [133]. This drug is a possible emerging treatment that is already in phase III study in other types of tumors [134]. Another drug that inhibits the activity of the Met receptor by inducing the degradation of β-catenin is Celecoxib [120]. However, recent evidence indicates that the combined treatment of Celecoxib with the usual chemotherapy did not improve overall survival or progression-free time in patients with CRC [135]

On the other hand, a large number of clinical trials have been developed using cytokine-based immunotherapy for CRC. Different drugs targeting IL, prostaglandins, CXCL, TNFα and TGF-β superfamily factors are in clinical trials. Although it has not yet been proven that they generate an effect as monotherapy or in combined treatments with commonly used drugs, future expectations are very optimistic [136–138].

The employing of non-coding RNA such as miRNAs or cRNAs is a promising solution to overcome CRC drug resistance. It has been seen that miR505, miR199a/b and miR320 decreased sensitivity to Cisplatin and OXA by modulating the activity of the Wnt/ β-catenin pathway. Others, such as miR30-5p, favor chemosensitivity in CRC cells [139]**.** In this work we have also described the role of TME-derived cRNAs in activating the β-catenin signaling pathway and its influence on CRC development and progression. Nevertheless, there is still a lot to explore about its potential role in future therapies for CRC. In this regard, recent research indicates that knockdown circ-PRKDC decreases the resistance of CRC-derived cells to 5-FU by modulating the Wnt/β-catenin pathway [80]. These results could postulate a new strategy to deal with multidrug resistance in CRC. Additionally,

Viralippurath and collaborators propose that gene therapy could be aimed at silencing these oncogenic cRNAs or increasing the effects of those molecules that act as tumor suppressors [27]. Despite these findings, to date, there are no available therapies that use these small molecules to evade the strategies of tumor cells against chemotherapeutic drugs. More studies are needed regarding the role of non-coding RNAs in CRC chemoresistance as well as for the development of delivery strategies for these molecules in new targeted therapies. **Figure 4** schematizes current studies related to disruption of the interrelationships between TME and CRC cells that trigger β-catenin activation and are involved in treatment failure.

As we have previously mentioned, autophagy is a mechanism associated with chemoresistance in CRC [109]. Recent investigations aim to find drugs that help reverse the autophagy process and overcome chemoresistance in CRC [140]. According to Pérez-Plasencia and colleagues, the involvement of Wnt/β-catenin signaling in the regulation of autophagy was demonstrated in several types of cancer, such as leukemia, hepatocarcinoma, squamous cell carcinoma, lung cancer and prostate cancer [64]. However, it is just a matter of time before the relationship between these two pathways in CRC will be considered crucial for future pharmacological treatments. In their work, the authors postulate several therapeutic approaches based on drugs that are capable of simultaneously disrupting components of β-catenin and autophagy pathways [64].

From what we have analyzed in this work, it is important to highlight that concerning CRC, the achievement of successful future therapies will involve the use of combined pharmacological compounds that inhibit not only the canonical β-catenin signaling pathway but also the TME- derived signaling"

Reviewer's comment 3.

Can the gut bacteria and their metabolites enriched in CRC's TME affect the Wnt/β-catenin pathway?

Author response to comment 3:

We appreciate the interesting question of the reviewer as it made us consider other aspects of the TME that we have omitted while writing our manuscript. We have reviewed the available published literature on the actions of various components of the gut microbiota and found that, in recent years, several bacteria have been closely related to key aspects of CRC development and progression through the Wnt/β- pathway catenin. On the other hand, we found information on how some probiotics can exert beneficial effects in the prevention of CRC through the negative modulation of the Wnt/β-catenin pathway. We found these research very interesting and have added this content to Section "**2. TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC**", in the last two paragraphs**,** as follows:

"Bacteria, fungi, and viruses constitute the microbiota residing within the gastrointestinal tract. However, the gut microbiota and their metabolites can be altered in the TME and directly influence on CRC progression [97,98] Various bacteria have been implicated in these processes by modulating β-catenin pathway. *Fusobacterium nucleatum* is known as a CRC inductor and suppressor of NK cells activity [97,99] Recently, it was reported that *F. nucleatum* can stimulate Annexin A1 protein, specifically expressed in CRC cells. Annexin A1 can then provocate β-catenin nuclear accumulation and finally the exacerbation of CRC cells proliferation [100]. On the opposite side, as extensively analyzed by Li and collaborators in their Review article, other bacteria could be beneficial for CRC treatment or its prevention. For instance, *Lactobacillus* and *Clostridium butyricum* are probiotics that have shown an inhibitory effect in CRC progression via modulation of the β-catenin pathway [97] This background evidences that the complex interrelations between tumor cells and their microenvironment (including the microbiota) involve the participation of one of the most outstanding signaling pathways in the promotion and development of CRC, such as the β-catenin pathway. Therefore, it is important to consider these phenomena in the development of new treatments that focus on inhibiting or silencing the activators/exacerbators of this pathway".

删除[Julia Martin]:

Reviewer's comment 4.

"Recently, studies have shown that these vesicles can carry several proteins, lipids". What proteins and lipids can be carried by vesicles?

Reviewer's comment 5.

The transfer of Wnt ligands or β-catenin via Extracellular Vesicles （EVs） has been proposed as a Wnt signalling activation mechanism. They have great impact on proliferation, motility, EMT, migration, invasion, immune evasion, chemoresistance, and TME reprogramming. A more detailed introduction to EVs may make the article more comprehensive.

Author response to comments 4 and 5:

The following responses respond to reviewer comments 4 and 5 as they are related.

Thank you for your observation. We have realized that we were very unspecific about the importance given to EVs related to CRC and we only analyzed a few works dealing with exosomes. According to the reviewer's suggestion, we have added a paragraph with the importance of EVS in CRC events such as metastasis, as well as a more detailed description of EVs nature. Furthermore, we were more explicit across the text on which biomolecules, such as nucleic acids (microRNAs, long non-coding RNAs and circular RNAs), are carried extracellularly by EVs throughout the tumor stroma. These changes are shown below:

"1. Dynamics established between the TME and CRC cells"

".... The TME cells and the neoplastic cells continuously secrete and internalize factors that modulate the CRC development and contribute to the maintenance of the tumor ecosystem. These extracellular signals are schematized in **Figure 2** and involve soluble proteins, insoluble proteins from the ECM, or compounds of variable nature loaded in vesicles [22]. **Table 1** compiles the biomolecules well described in the literature that are secreted by the TME cells that allow communication with CRC cells. Some of these factors can travel freely through the tumor stroma space, while others are transported through membrane-bound vesicles known as extracellular vesicles (EVs) [23,24]. EVs structure includes a lipid bilayer composed mainly of ceramide, cholesterol, sphingomyelin, phosphoglycerides,

glycosphingolipids, phosphatidyl serine, phosphatidylethanolamine, mannose, N-linked glycans, polylactosamine and sialic acid [23]. In recent years, EVs have acquired relevance as biological mediators in the communication between the TME and tumor cells. Concerning EVs related to CRC, their loads are considered prognostic factors or indicative of response to therapies. Recent studies have shown that EVs can carry large amounts of biomolecules from TME cells to cancer cells and vice versa. Usual cargoes include cytokines/chemokines, angiogenic factors, extracellular matrix remodeling factors and nucleic acids such as microRNAs (miRs), long non-coding RNAs (lncRNAs) and circular RNAs (cRNAs) [25–28]. It has been shown that fluids from CRC patients, such as plasma, saliva, urine, etc, contain large amounts of secreted EVs. These molecules can stimulate or inhibit the expression of oncogenes and oncoproteins, affecting the phenotype of the neoplastic cells or altering the secretory profile of the TME cells [23,26,29]. Unlike cytokines and growth factors that act in the nearby cells, the EVs can influence both the primary colorectal tumor and the metastatic niche remotely [23,30]. For instance, the analysis of the plasma from CRC patients revealed that EVs are directly linked to the establishment of liver proinflammatory phenotype and liver metastasis, being this effect mediated by modulation of macrophages by EVs [31]. Correlation between CD8+ T cells activation and EVs high concentrations in plasma has also been demonstrated, thus evidencing the role of EVs in the modulation of the immune response in CRC [32]. In conclusion, EVs become a new source of therapeutic targets for CRC [23]…"

"**2. TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC"**

"**…**EVs have also shown a key role in activating Wnt signaling in CRC. On the one hand, EVs can transport mutant β-catenin and activate the Wnt signaling pathway in the recipient cells, thus promoting CRC progression [58]. Regarding the effects of EVs concerning the TME cells, Hu and colleagues have demonstrated *in vitro* that fibroblast-derived exosomes (one type of EVs) promoted the tumor growth of CSCs upon treatment with 5-fluorouracil (5-Fu) or oxaliplatin (OXA) [59]. Further investigations of the same group in subcutaneous xenografts achieved through HT-29 CRC-derived cells and fibroblasts co-implantation demonstrated that stromal fibroblasts can secrete exosomes loaded with Wnt ligands. Once these exosomes interact with differentiated CRC cells, Wnt ligands induce a phenotypic

reversion of CRC cells to CSCs, which includes features such as the expression of CSCs markers and elevated Wnt activity [60].

Hypoxia is a prevalent condition in the solid tumors TME, which involves oxygen pressures of less than 5-10 mmHg. Hypoxic TME can deeply influence CRC, and these effects may be mediated by β-catenin. For instance, it was found *in vitro* that Wnt/β-catenin signaling is responsible for the hypoxia-induced self-renewal of colorectal-stem cells [61]. Furthermore, Huang and collaborators observed that hypoxic CRC cells, can secrete exosomes enriched with Wnt4 ligands. These exosomes can activate β-catenin signaling in normoxic CRC cells and stimulate prometastatic behaviors such as cell migration and invasion [62]. In another work, they demonstrated that Wnt4-loaded exosomes secreted by the tumor cells promoted angiogenesis through the proliferation and migration of endothelial cells. These effects were induced in conditions of hypoxia and mediated by the activation of Wnt/β-catenin signaling [63]…"

"… Tumor small and long non-coding RNAs are other factors transported by EVs, that are also involved in the modulation of β-catenin signaling…"

We also added EVs to **Figure 1** of this letter (**Figure 2** of the revised manuscript).

Figure 1

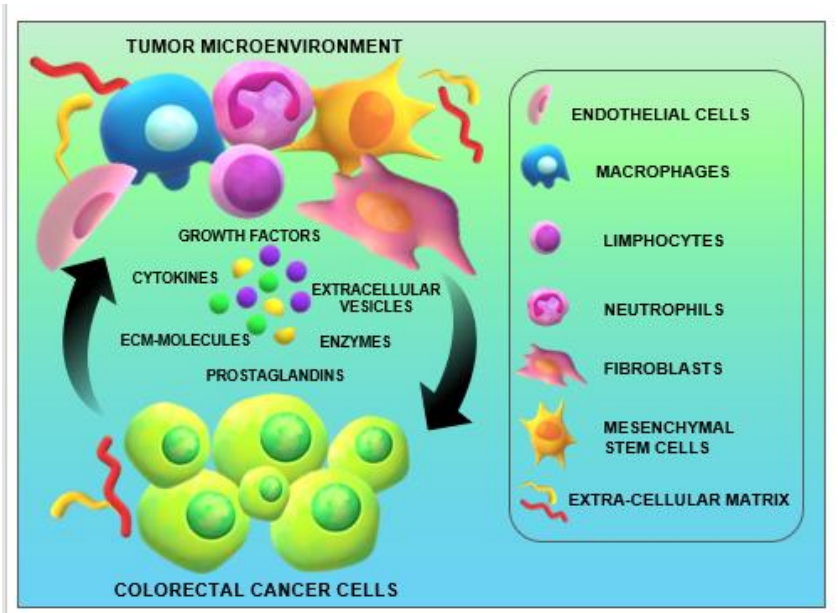

Figure 2. Extracellular signal molecules secreted by TME and neoplastic cells. The traffic of soluble and insoluble factors allows crosstalk between tumor cells and their environment modulating CRC development and progression. This figure is original for this work.

Reviewer's comment 6.

TLR signaling wide expression within the TME of CRC, but TLR signaling did not shown in the Table 1.

Author response to comment 6:

We appreciate the valuable observation of the reviewer. We have included TLR signaling in **Table 1**. Moreover, we have now incorporated content on the involvement of TLR signaling in CRC genesis and progression to Section "**1. Dynamics established between the TME and CRC cells",** as follows:

"…Other proteins with different functions were studied in relation to CRC and the TME. In recent years, it has been reported that both the neoplastic cells and the TME cells can synthesize and secrete enzymes that participate in tumorigenesis. Muñoz-Galván and colleagues demonstrated that phospholipase D2 (PLD2) enzyme is overexpressed and

secreted by CRC cells. PLD2 in the extracellular space modulates the phenotype and secretory profile of CAFs and thus contributing to promoting stemness in tumor cells [39]. Other enzymes like nitric oxide synthase (iNOS) are capable of inducing immunomodulatory effects [40]. Moreover, enzymes present in the TME, such as metalloproteases (MMP), enable the invasion and migration of cancer cells [40]. These enzymes act mainly by altering the structure and function of the ECM, thus allowing the activation of other factors that amplify the pro-tumor signals [41]. Receptor proteins such as Toll-like receptors (TLRs) play a fundamental role in maintaining epithelial barrier homeostasis in the gut and mediating inflammatory and immune responses [42]. Today, it is known that TLRs are also expressed in fibroblasts and ECs in the TME and in CRC cells. The dysregulation in TRLs pathways is associated with inflammation-driven carcinogenesis [43]. In response to this evidence, currently, there are clinical trials that include TLRs agonists for the treatment of CRC (See www.clinicaltrials.gov) [42,44].

The above-mentioned factors generally exert their effects by modulating several signaling pathways in the tumor [1,45–47]. In accordance with the focus of this contribution, in the next Sections, we will further discuss which of these biomolecules participate in the aberrant activation of the β-catenin signaling pathway in CRC, and how these findings have provided new opportunities for the development of more efficient therapies…"

Reviewer's comment 7.

Revise the reference format of the sixth line from the bottom on page 6.

Author response to comment 7:

We have checked all the references format to correct mistakes.

Reviewer's comment 8.

Hypoxia is a key feature of tumor microenvironment, authors need to add some relevant studies about the correlation between Wnt/ β- catenin signaling pathway and hypoxia signaling pathway.

Author response to comment 8:

We appreciate the valuable observation of the reviewer. We have now added some studies about the correlation between Wnt/ β- catenin signaling pathway and hypoxia in the CRC TME. We have also related this parameter to autophagy. These changes are included in Section "**2. TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC"**, as shown below:

"…Hypoxia is a prevalent condition in the solid tumors TME, which involves oxygen pressures of less than 5-10 mmHg. Hypoxic TME can deeply influence CRC, and these effects may be mediated by β-catenin. For instance, it was found *in vitro* that Wnt/β-catenin signaling is responsible for the hypoxia-induced self-renewal of colorectal-stem cells [61]. Furthermore, Huang and collaborators observed that hypoxic CRC cells, can secrete exosomes enriched with Wnt4 ligands. These exosomes can activate β-catenin signaling in normoxic CRC cells and stimulate prometastatic behaviors such as cell migration and invasion [62]. In another work, they demonstrated that Wnt4-loaded exosomes secreted by the tumor cells promoted angiogenesis through the proliferation and migration of endothelial cells. These effects were induced in conditions of hypoxia and mediated by the activation of Wnt/β-catenin signaling [63].

Autophagy is a physiological process through which normal cells degrade intracellular components to keep cellular homeostasis. It is postulated as an alternative to cell death when the apoptotic machinery fails and is associated both with CRC and the TME, with controversial findings [64–66]. TME hypoxia has been shown to induce autophagy and also activates several tumor escape mechanisms [65]. Some of the molecular mechanisms involved in the modulation of autophagy are Phosphoinositide 3-kinases (PI3K)/ Protein kinase B (also known as Akt)/mammalian target of rapamycin (mTOR), TGF- β, Notch, and the Wnt/β-catenin signaling [66]. According to evidence, autophagy induced in the early stages of tumor development prevents the infiltration of immune cells as well as tumor cell death. Autophagy can also promote tumor progression in advanced stages of the disease [66]. On the other hand, it has been reported that the relationship between autophagy and increased lymphocytic infiltration in the TME is mediated by the

autophagy-related protein Vps34 in CRC. The modulation of this autophagy-related protein would improve the efficacy of immunotherapies [67]. However, it is still necessary to deepen the study on the interrelation between the Wnt/β-catenin signaling pathway and autophagy, and which TME factors could mediate these events in CRC…"

Reviewer's comment 9. The research on the influence of tumor microenvironment on chemoresistance has attracted great attention. At present, there are similar articles to summarize this content. This paper only includes the recent researches, authors does not put forward new ideas and lack of novelty.

Author response to comment 9: We agree with the reviewer in that there are several articles dealing with similar topics. However, from our search in databases, the only review paper that addresses the issue of the TME relationship with CRC cells through Wnt/ β-catenin modulation was published in 2008 (Huang D, Du X. Crosstalk between tumor cells and microenvironment via Wnt pathway in colorectal cancer dissemination. World J Gastroenterol 2008; 14: 1823. [DOI: 10.3748/wjg.14.1823]). As a matter of chronology and improved research technologies, the discovery and association of new molecules and factors to CRC has increased considerably, as well as the great advance in what is known regarding the TME-CRC relationship. We have now further explained our aim in the last sentence in the Introduction section and in the Abstract (see below). We hope that after attending to all the valuable suggestions of the reviewers, you will consider our manuscript recommended for publication.

"ABSTRACT

Colorectal cancer (CRC) continues to be one of the main causes of death from cancer because patients progress unfavorably due to resistance to the current therapies. It is well established that dysregulation of the Wnt/β-catenin pathway plays a fundamental role in the genesis and progression of several types of cancer, including CRC. In many subtypes of CRC, hyperactivation of the β-catenin pathway is associated with mutations of the APC gene. However, it can also be associated with other causes. In recent years, studies of the tumor microenvironment (TME) have demonstrated its importance in the development and

progression of CRC. In this tumor nest, several cells types, structures and biomolecules are interacting with the neoplastic cells to pave the way for the spread of the disease. Cross-communications between tumor cells and the TME are then established primarily through paracrine factors, which trigger the activation of numerous signaling pathways. Crucial advances in the field of oncology have been made in the last decade. This Minireview aims to actualize what is known about the central role of the Wnt/β-catenin pathway in relation to CRC chemoresistance and aggressiveness, focusing on the cross-communication between the CRC cells and the TME. Through this analysis, our main objective is to increase the understanding of this complex disease considering a more global context. Since many treatments for advanced CRC fail due to mechanisms involving chemoresistance, the data here exposed and analyzed are of great interest for the development of novel and effective therapies."

"INTRODUCTION"

"…This work aims to analyze the available literature regarding the biomolecules associated with the TME that can modulate the Wnt/β-catenin signaling pathway in CRC cells, which allows a better understanding of the biological causes of the high morbidity and mortality of this pathology in a global context. Although antecedents of this particular analysis are registered in 2008 [20], the enormous amount of information collected in recent years results in the emergence of new biomolecules, structures and even processes related to TME and CRC cells that were previously unknown or unrelated. The comprehension of this information enables countless new possibilities for the development of new therapies for CRC…"

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

How many patients did you examine in your study?

Author response:

Dear Reviewer, our previous published studies on the effects of Parathyroid Hormone-related Peptide in colorectal cancer (CRC) were conducted entirely *in vitro* (in human CRC-derived cell lines HCT116 and Caco-2) and *in vivo* in xenografts. The latter was achieved by inoculating HCT116 cells s.c. on the flanks of athymic ("nude") mice. The obtained results are included in the following 7 articles:

-"Involvement of Parathyroid Hormone Related Peptide in the aggressive phenotype of colorectal cancer cells", (2021). Belén Novoa Díaz, Pedro Matías Carriere, María Julia Martín, Natalia Calvo, Claudia Gentili. World Journal of Gastroenterology: 27(41): 7025-7040. DOI: 10.3748/wjg.v27.i41.7025.

-"Role of SPARC in the epithelial-mesenchymal transition induced by PTHrP in human colon cancer cells", (2021) Pedro Carriere , Natalia Calvo , María Belén Novoa Díaz , Fernanda Lopez-Moncada , Alexander Herrera, María José Torres, Exequiel Alonso, Norberto Ariel Gandini, Graciela Gigola, Hector R Contreras, Claudia Gentili. Molecular and Cellular Endocrinology. 15; 530:111253. DOI: 10.1016/j.mce.2021.111253.

Carriere Pedro, Novoa Díaz María Belén, López Moncada Fernanda, Zwenger Ariel, Contreras Héctor, Calvo Natalia, Gentili Claudia. *PTHrP and SPARC expressions in human colorectal cancer: An in silico analysis*. Annals of Oncology. 2021. 32:158. https://doi.org/10.1016/j.annonc.2021.05.227

María belén Novoa Díaz, Ariel Zwenger, Carriere pedro, Martín María Julia, Calvo Natalia, Gígola Graciela, Gómez Luis, Gentili Claudia. *Molecular mechanisms related to chemoresistance of colorectal cancer cells.* Annals of Oncology. 2020. 31-1230. http://dx.doi.org/10.1016/j.annonc.2020.08.2202

-"PTHrP treatment of colon cancer cells promotes tumor associated angiogenesis by the effect of VEGF", (2019). Natalia Calvo, Pedro Carriere, María Julia Martín, Claudia Gentili. Molecular and Cellular Endocrinology, 483:50-63. DOI: 10.1016/j.mce.2019.01.005.

-“Potential therapeutic targets for growth arrest of colon cancer cells exposed to PTHrP”, (2018) María Julia Martín, Graciela Gigola, Ariel Zwenger, Martin Carriquiborde, Florencia Gentil, Claudia Gentili. Molecular and Cellular Endocrinology: 478: 32-44. DOI: 10.1016/j.mce.2018.07.005.

“RSK activation via ERK modulates human colon cancer cells response to PTHrP” (2017), Natalia Calvo, Carriere Pedro, M. Julia Martin, Claudia Gentili. Journal of Molecular Endocrinology 59: 13-27. DOI: 10.1530/JME-16-0216.

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-“Involvement of ERK 1/2, p38 MAPK and PI3k/Akt signaling pathways in the regulation of cell cycle progression by PTHrP in colon adenocarcinoma cells” (2014), Natalia Calvo, M. Julia Martin, Ana Russo de Boland, Claudia Gentili. Biochemistry and Cell Biology, 92(4): 305-315. DOI: 10.1139/bcb-2013-0106.

Notwithstanding, we have recently had access to samples of patients with CRC and their medical records, and we performed immunohistochemical analysis to establish a correlation between tumor microenvironment factors, such as PTHrP and Met (Hepatocyte Growth Factor receptor), and tumor aggressiveness and progression. The data resulting from this analysis are included in an article currently under review.

For the reviewer information:

To satisfy the suggestion of all reviewers, we have deleted Sections 1 and 2 of the original manuscript and the summarized content of them was included in the Introduction section and in section “TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC” (section 4 of the original manuscript). Also, we have now merged section 3 and section 4 of the original manuscript.

According to the information included in the MiniReview, we considered now reformulating the following section title:

"TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC"

Instead of:

"TME factors that modulate the Wnt/β-catenin pathway in CRC"

Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors:

In this review, the author described the cross-communication between Wnt/β-catenin pathway and cancer microenvironment in carcinogenesis, progression, and chemoresistance in CRC. It is a comprehensive review, and the prospective sight is the development of the pathway inhibitor in the disease.

Reviewer's comment 1.

In the introduction, I think the more precise description of CRC, tumor microenvironment, and the pathway is better in the introduction.

Author response to comment 1:

Thank you for your advice. We agreed with the reviewer and have described more precisely the basis of CRC, the tumor microenvironment and the Wnt/β-catenin pathway in the Introduction section, as follows:

"**INTRODUCTION**

Colorectal cancer (CRC) is the third most frequent malignant disease worldwide in both men and women [1,2]. Data provided by the World Cancer Research Fund International indicate that CRC incidence is growing, and the prevalence of this pathology is expected to increase by 60% in the next 15 years [3]. Among all cancer-induced deaths, the ones

associated with CRC reach values of 8-9% [4,5]. These statistics rely mainly on the fact that 20% of patients show metastasis (stage IV) at the time of diagnosis and the overall survival in these patients is low due to failure in the treatments [5,6]. To improve the response of these patients to therapy, it is necessary to expand the knowledge about the mechanisms that play a critical role in the development, progression and chemoresistance of CRC.

In 2015, Guinney and collaborators proposed a classification of colorectal tumors based on four consensus molecular subtypes (CMSs). Groups were then defined according to certain parameters such as clinical, genetic and molecular characteristics (see **Figure 1**): CMS1 group, also denominated MSI Immune, is characterized by high microsatellite instability and immune response. The Canonical subtype (CMS2) represents 37% of cases and presents hyperactivation of the Wnt and Myc signaling pathways. The CMS3 (Metabolic subtype) is characterized by a marked dysregulation of metabolic pathways. Finally, the CMS4 (Mesenchymal subtype) group exhibits hyperactivation of the transforming growth factor-β (TGF-β) pathway and a marked transition from epithelial to mesenchymal phenotype (EMT program) [7–9]. Remarkably, 90% of CRC presents aberrant activation of the canonical Wnt/β-catenin signaling pathway [10–12]. This pathway is strictly regulated in physiological conditions and modulates fetal development and homeostasis in adult tissues [12]. Briefly, in the absence of Wnt ligand, β-catenin is continuously phosphorylated in the cytoplasm by a destruction complex comprised of Adenomatous polyposis coli (APC), Axis inhibition protein (AXIN), Glycogen synthase kinase 3 (GSK3) and Casein kinase 1 (CK1). Once phosphorylated, β-catenin is ubiquitinated and degraded by the proteasome. When the Wnt ligand binds to its receptor, the destruction of β-catenin stops, so this protein accumulates in the cytosol and then translocates to the nucleus. Finally, β-catenin binds to transcription factors from TCF/LEF family and promotes the transcription of several genes [13]. There are several published research as well as Review articles on the involvement of the Wnt/β-catenin pathway in CRC development, progression and chemoresistance [11,14,15]. Furthermore, this pathway became an unquestionable target for novel therapies for CRC. Despite this, to date, there are no approved treatments or clinical trials for CRC based on targeting the Wnt/β-catenin pathway [16].

As extensively described in the open literature, the hyperactivation of the Wnt/β-catenin pathway in CRC is directly associated with intrinsic causes such as mutations in the *APC (adenomatous polyposis coli)* gene. This results in the nuclear accumulation of β-catenin and the induction of Wnt target genes that promote tumor progression [17]. In this contribution, we intend to analyze how Wnt/β-catenin aberrant activation can be induced or exacerbated by external causes.

In the last decade, the tumor microenvironment (TME) or tumor stroma has become relevant in the progression of CRC [18]. The TME is a niche composed of the extracellular matrix, a great variety of modified stromal cells and aberrant vasculature [10]. Intercellular communications between the tumor cells and the TME are mainly established through paracrine signaling [10,19]. These communications and factors are extrinsic and may directly affect CRC progression through the Wnt/β-catenin pathway. In this way, a range of new therapeutic targets appears, expanding the possibilities of achieving effective treatments for CRC.

This work aims to analyze the available literature regarding the biomolecules associated with the TME that can modulate the Wnt/β-catenin signaling pathway in CRC cells, which allows a better understanding of the biological causes of the high morbidity and mortality of this pathology in a global context. Although antecedents of this particular analysis are registered in 2008 [20], the enormous amount of information collected in recent years results in the emergence of new biomolecules, structures and even processes related to TME and CRC cells that were previously unknown or unrelated. The comprehension of this information enables countless new possibilities for the development of new therapies for CRC. "

Reviewer's comment 2.

Point 2, is it ok for illustrating the cross-communication between Wnt/β-catenin pathway and cancer microenvironment in colorectal carcinogenesis?

Point 3 and 4 can be combined as an integral part demonstrating integration of them in CRC progression. The most important is the application of pathway inhibitor in CRC, I think this part can be considered as an important section in the review. The author shall be more comprehensively described the development and usage of the inhibitor in CRC, the clear mechanism diagram shall be provided.

Author response to comment 2:

We appreciate the reviewer's observation. Based on the Reviewer's comment, we have now improved **Figure 3** of the revised manuscript (**Figure 2** of this letter). We also completed this figure with TME events that participates in the Wnt/β-catenin pathway activation in CRC.

To satisfy the suggestion of all reviewers, we have deleted Sections 1 and 2 of the original manuscript and the summarized content of them was included in the Introduction section and in section "2. TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC" (section 4 of the original manuscript). Also, we have now merged section 3 and section 4 of the original manuscript. According to the information included in the MiniReview, we considered now reformulating the following section title:

"2.TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC"

Instead of:

"TME factors that modulate the Wnt/β-catenin pathway in CRC"

Finally, more content on the new therapeutic approaches for CRC based on disrupting Wnt/β-catenin pathway and the importance of immune evasion has been added (please see sections 3 and 4 of the revised manuscript). Moreover, we added in this Section a new figure (**Figure 4** of the revised manuscript, **Figure 3** of this letter) with the canonical activation of Wnt/β-catenin pathway, TME signaling and the pharmacological targets that are currently under study to block TME signals that activate the Wnt/β-catenin pathway activation in CRC.

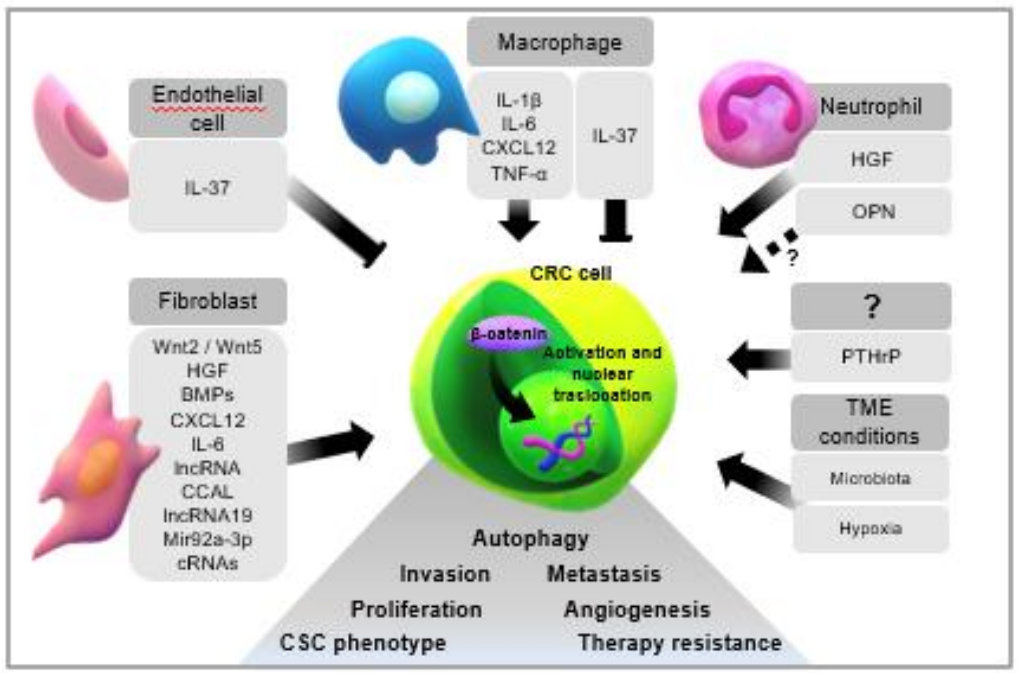

Figure 2.

Figure 2. Influence of TME-derived factors in the activation of β-catenin pathways. Several cytokines, growth factors, and small nuclei acid molecules secreted by stromal cells induce aberrantly activation of β-catenin and its nuclear translocation in CRC cells promoting events associated with an aggressive phenotype of the tumor cells. Il-37 represses the expression of β-catenin and its transcriptional activity. OPN is statistically associated with the expression of β-catenin in CRC and is known to induce its activation in other types of cancer. More studies are necessary to confirm positive feedback between the secretion of this TME factor and β-catenin signaling pathways in CRC. In addition to these factors, the microbiota and the hypoxia in the TME, also participate in the modulation of β-catenin activation. This figure is original for this work. BMP, Bone Morphogenic Protein; cRNA, circular RNA; CCAL, colorectal cancer-associated lncRNA; CRC, colorectal cancer; CXCL, C-X-C motif chemokine ligand; HGF, Hepatocyte Growth Factor; IL, Interleukin; lncRNA, long non-coding RNA; OPN, Osteopontin; PTHrP, Parathyroid Hormone-related Peptide; TNF-α, Tumor Necrosis Factor alfa; Wnt, Wingless protein.

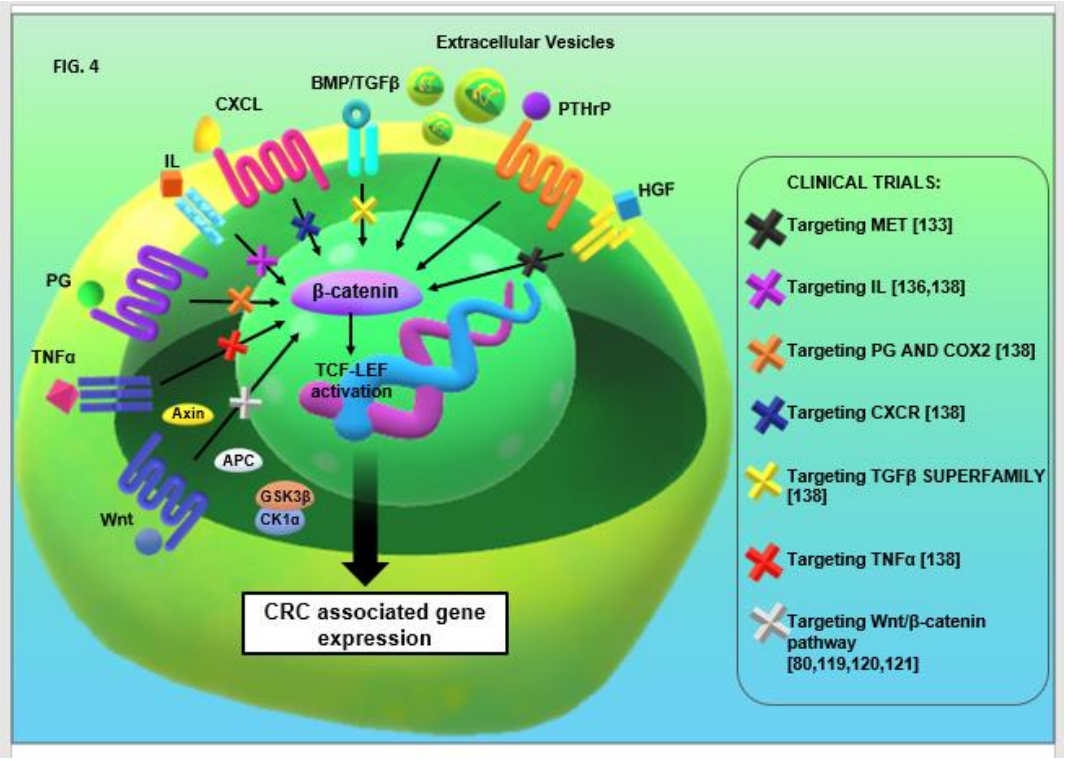

Figure 3.

Figure 3. Pharmacological targeting of the TME factors involved in the activation of β-catenin signaling in CRC. Several therapeutic strategies to block or inhibit the induction of β-catenin pathway by TME factors in CRC are currently under clinical study. This figure is original for this work. APC; Adenomatous polyposis coli; BMP, Bone Morphogenic Protein; CK1α; Casein kinase 1 alpha; CRC, colorectal cancer; CXCL, C-X-C motif chemokine ligand; GSK3β, Glycogen Synthase Kinase 3 Beta; HGF, Hepatocyte Growth Factor; IL, Interleukin; PTHrP, Parathyroid Hormone-related Peptide; PG, prostaglandin; TCF/LEF, T-cell factor/lymphoid enhancer factor; TGF-β, transforming growth factor-beta; TNF-α, Tumor Necrosis Factor alfa; Wnt, Wingless protein.

Since we updated the information on pharmacological targets in Section 4 of the revised manuscript, we consider it pertinent to add data recently obtained in our laboratory, as follows:

"In our laboratory, we have found that the selective inhibition of the HGF receptor, the RTK Met, suppresses β-catenin phosphorylation at domains that favor its nuclear translocation induced by PTHrP (Novoa Diaz et al., unpublished work)."

The reviewer can see the results in **Figure 4** of this letter.

Figure 4.

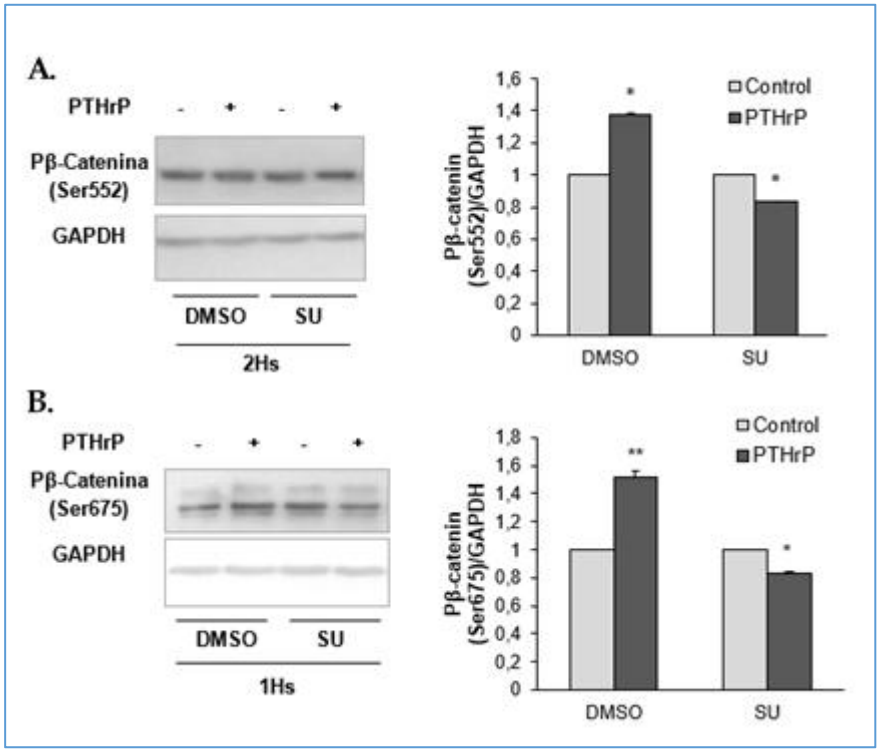

Figure 4. Met receptor mediates β-catenin phosphorylation and activation induced by PTHrP in the HCT116 cell line. Cells were pre-incubated with SU11274, a specific Met inhibitor and then treated with or without PTHrP (10^{-8}M). The protein levels of (A) pβ-catenin (Ser552) and (B) pβ-catenin (Ser675) were analyzed by Western Blot to investigate whether the molecular mechanisms triggered by PTHrP are capable of modulating the phosphorylation and activation of β-catenin through Met signaling. GAPDH protein levels were determined as control of the amount of proteins present in the membrane since this protein is not substantially modified with the treatment by the cytokine. Graph bars represent the average of the results obtained from three independent experiments. $^{*}p < 0.05$; $^{**}p < 0.01$. DMSO, Dimethyl sulfoxide; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; PTHrP, Parathyroid Hormone-related Peptide; Ser, serine; SU, SU11274.

Reviewer #4:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

I've read with great interest the paper "Tumor microenvironment involvement in colorectal cancer progression via Wnt/β-catenin pathway: providing understanding of the complex mechanism of chemoresistance" by María B Novoa Díaz et al. It is a very ehaustively elaborated review regarding the complex topic the authors dealed with, that I will hardly call as a mini review.

Reviewer's comment 1.

The paper is quite long. All the molecular mechanism involved in the topic are evocated and of course this needs a lot of space. I would suggest to reduce the point 2 to just the last part starting with I 2015.

Author response to comment 1:

We agree with the Reviewer. We have reduced the information on CRC classification, and this was integrated into the Introduction Section in order to summarize the Minireview.

Reviewer's comment 2.

Probably the point 3 also may be included in point 4 and reduced to just one sentence. There are some abbreviations that are not mentioned in the table.

Author response:

Thank you for your advice. To satisfy the suggestion of all reviewers, we have deleted Sections 1 and 2 of the original manuscript and the summarized content of them was included in the Introduction section and in Section 2 "TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC" (section 4 of the original manuscript). Also, we have now merged section 3 and section 4 of the original manuscript.

We also completed the abbreviations missed in **Table 1** of the revised manuscript.

Reviewer's comment 3.

The english has to be reviewed. Summarizing, the paper is very complex and offers a detailed vision on the mollecular mechanisms involved in colorectal cancer evolution and their impliction on the oncology treatment. However, it is very extensive so an effort to summarise the text would be of great benefit for the readers.

Author response:

We appreciate the valuable comments of the reviewer. We made an effort to summarize our contribution, and at the same time added more content suggested by other reviewers (including immune evasion, extracellular vesicles, autophagy, hypoxia, etc.) which greatly improved our work.

For the Reviewer information: According to the information included in the MiniReview, we considered now reformulating the following section title:

2. TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC

Instead of:

TME factors that modulate the Wnt/β-catenin pathway in CRC

We hope that after all these modifications you will find our Mini-Review recommendable for publication.

Reviewer #5:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

Comments to the authors The article with the title "Tumor microenvironment involvement in colorectal cancer progression via Wnt/β-catenin pathway: providing understanding of the complex mechanism of chemoresistance" is well done, but I would offer these comments to the investigators:

Reviewer's comment 1. Several words throughout the manuscript appear to be merged. Please correct it.

Autor response to comment 1:

We appreciate the reviewer's observation. We have checked the entire manuscript to correct these mistakes.

Reviewer's comment 2.

Some minor grammatical errors occur. The manuscript contains significant language-related issues. Please correct these types of grammatical errors throughout the paper.

Author response to comment 2:

Thank you for the reviewer´s advice. We have overall improved the language in the manuscript.

Reviewer's comment 3.

It will be an interesting topic for your work to mention the association of Wnt/β-catenin pathway with autophagy. Autophagy is a basic catabolic process with TME development and the cross-presentation of neo-antigen.

Author response to comment 3:

We agreed with the reviewer that autophagy should be considered in our mini-review. Accordingly, we have added autophagy in the Figure 3 of the revised manuscript, and have included content on the effects of autophagy in CRC in relation to the tumor microenvironment and the chemoresistance via the Wnt/β-catenin pathway, as follows:

"**2. TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC**"

"...Autophagy is a physiological process through which normal cells degrade intracellular components to keep cellular homeostasis. It is postulated as an alternative to cell death when the apoptotic machinery fails and is associated both with CRC and the TME, with controversial findings [64–66]. TME hypoxia has been shown to induce autophagy and also activates several tumor escape mechanisms [65]. Some of the molecular mechanisms involved in the modulation of autophagy are Phosphoinositide 3-kinases (PI3K)/ Protein kinase B (also known as Akt)/mammalian target of rapamycin (mTOR), TGF- β, Notch, and the Wnt/β-catenin signaling [66]. According to evidence, autophagy induced in the early stages of tumor development prevents the infiltration of immune cells as well as tumor cell death. Autophagy can also promote tumor progression in advanced stages of the disease [66]. On the other hand, it has been reported that the relationship between autophagy and increased lymphocytic infiltration in the TME is mediated by the autophagy-related protein Vps34 in CRC. The modulation of this autophagy-related protein would improve the efficacy of immunotherapies [67]. However, it is still necessary to deepen the study on the interrelation between the Wnt/β-catenin signaling pathway and autophagy, and which TME factors could mediate these events in CRC…"

"3. Role of β-catenin and the TME in the chemoresistance of CRC "

"…It was also shown that CAFs are capable of inducing drug resistance by transferring small nucleic acid molecules such as H19 or CCAL lncRNA and activating β-catenin in neighboring cells [26]. H19 lncRNA can also trigger autophagy via SIRT1 and consequently induce resistance to 5-FU in CRC cells [109]…"

"4. The TME and β-catenin pathway: future perspectives for CRC management"

"...As we have previously mentioned, autophagy is a mechanism associated with chemoresistance in CRC [109]. Recent investigations aim to find drugs that help reverse the autophagy process and overcome chemoresistance in CRC [140]. According to Pérez-Plasencia and colleagues, the involvement of Wnt/β-catenin signaling in the regulation of autophagy was demonstrated in several types of cancer, such as leukemia, hepatocarcinoma, squamous cell carcinoma, lung cancer and prostate cancer [64]. However, it is just a matter of time before the relationship between these two pathways in CRC will

be considered crucial for future pharmacological treatments. In their work, the authors postulate several therapeutic approaches based on drugs that are capable of simultaneously disrupting components of β-catenin and autophagy pathways [64].

From what we have analyzed in this work, it is important to highlight that concerning CRC, the achievement of successful future therapies will involve the use of combined pharmacological compounds that inhibit not only the canonical β-catenin signaling pathway but also the TME- derived signaling…"

For the reviewer information:

To satisfy the suggestion of all reviewers, we have deleted Sections 1 and 2 of the original manuscript and the summarized content of them was included in the Introduction section and in section titled "TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC" (section 4 of the original manuscript). Also, we have now merged section 3 and section 4 of the original manuscript.

According to the information included in the MiniReview, we considered now reformulating the following section title:

"2. TME factors and conditions that modulate the Wnt/β-catenin pathway in CRC"

Instead of:

"TME factors that modulate the Wnt/β-catenin pathway in CRC"

Reviewer's comment 4.

Many references are considered old, and it is needed to be updated

Author response to comment 4.

To satisfy the Reviewer's concern, we have updated the references. They are listed below along with the newly added references corresponding to the content added to the manuscript.

Updated references:

Cox TR. The matrix in cancer. *Nat Rev Cancer* [Internet] 2021;**21**:217–38 [DOI: 10.1038/s41568-020-00329-7]Available from: http://www.nature.com/articles/s41568-020-00329-7

Instead of:

Hanahan D, Coussens LM. Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment. *Cancer Cell* 2012; **21**: 309–322. [DOI: 10.1016/j.ccr.2012.02.022]

Shah K, Kazi JU. Phosphorylation-Dependent Regulation of WNT/Beta-Catenin Signaling. *Front Oncol* [Internet] 2022;**12** [DOI: 10.3389/fonc.2022.858782]Available from: https://www.frontiersin.org/articles/10.3389/fonc.2022.858782/full

Instead of:

Taurin S, Sandbo N, Qin Y, Browning D, Dulin NO. Phosphorylation of β-Catenin by Cyclic AMP-dependent Protein Kinase. *J Biol Chem* 2006; **281**: 9971–9976. [DOI: 10.1074/jbc.M508778200]

Malki A, ElRuz RA, Gupta I, Allouch A, Vranic S, Al Moustafa A-E. Molecular Mechanisms of Colon Cancer Progression and Metastasis: Recent Insights and Advancements. *Int J Mol Sci* [Internet] 2020;**22**:130 [DOI: 10.3390/ijms22010130]Available from: https://www.mdpi.com/1422-0067/22/1/130

Instead of:

Wang J, Du Y, Liu X, Cho WC, Yang Y. MicroRNAs as Regulator of Signaling Networks in Metastatic Colon Cancer. *Biomed Res Int* 2015; **2015**: 1–12. [DOI: 10.1155/2015/823620]

Chu J, Fang X, Sun Z, Gai L, Dai W, Li H, Yan X, Du J, Zhang L, Zhao L, Xu D, Yan S. Non-Coding RNAs Regulate the Resistance to Anti-EGFR Therapy in Colorectal Cancer. *Front Oncol* [Internet] 2022;**11** [DOI: 10.3389/fonc.2021.801319]Available from: https://www.frontiersin.org/articles/10.3389/fonc.2021.801319/full

Instead of:

Wang J, Du Y, Liu X, Cho WC, Yang Y. MicroRNAs as Regulator of Signaling Networks in Metastatic Colon Cancer. *Biomed Res Int* 2015; **2015**: 1–12. [DOI: 10.1155/2015/823620]

Su K, Yi Q, Dai X, Liu O. Circular RNA ITCH: An Emerging Multifunctional Regulator. *Biomolecules* 2022; **12**: 359. [DOI: 10.3390/biom12030359]

Instead of:

Huang G, Zhu H, Shi Y, Wu W, Cai H, Chen X. cir-ITCH Plays an Inhibitory Role in Colorectal Cancer by Regulating the Wnt/β-Catenin Pathway. *PLoS One* 2015; **10**: e0131225. [DOI: 10.1371/journal.pone.0131225]

Yi J, Liu Y, Zhang L, Fang C. Secreted phosphoprotein-1 accelerates the progression of human colorectal cancer through activating β-catenin signaling. *Oncol Lett* 2021; **21**: 372. [DOI: 10.3892/ol.2021.12633]

Instead of:

Robertson BW, Chellaiah MA. Osteopontin induces β-catenin signaling through activation of Akt in prostate cancer cells. *Exp Cell Res* 2010; **316**: 1–11. [DOI: 10.1016/j.yexcr.2009.10.012]

Goïta AA, Guenot D. Colorectal Cancer: The Contribution of CXCL12 and Its Receptors CXCR4 and CXCR7. *Cancers (Basel)* 2022; **14**: 1810. [DOI: 10.3390/cancers14071810]

Instead of:

Song Z-Y, Gao Z-H, Chu J-H, Han X-Z, Qu X-J. Downregulation of the CXCR4/CXCL12 axis blocks the activation of the Wnt/β-catenin pathway in human colon cancer cells. *Biomed Pharmacother* 2015; **71**: 46–52. [DOI: 10.1016/j.biopha.2015.01.020]

Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol* 2017; **14**: 611–629. [DOI: 10.1038/nrclinonc.2017.44]

Erin N, Grahovac J, Brozovic A, Efferth T. Tumor microenvironment and epithelial mesenchymal transition as targets to overcome tumor multidrug resistance. *Drug Resist Updat* 2020; **53**: 100715. [DOI: 10.1016/j.drup.2020.100715]

Instead of:

Song M, Zang W, Zhang B, Cao J, Yang G. GCS overexpression is associated with multidrug resistance of human HCT-8 colon cancer cells. *J Exp Clin Cancer Res* 2012; **31**: 23. [DOI: 10.1186/1756-9966-31-23]

Hwang K, Yoon JH, Lee JH, Lee S. Recent Advances in Monoclonal Antibody Therapy for Colorectal Cancers. *Biomedicines* 2021; **9**: 39. [DOI: 10.3390/biomedicines9010039]

Instead of:

Woo JK, Kang J-H, Kim B, Park BH, Shin K-J, Song S-W, Kim JJ, Kim H-M, Lee S-J, Oh SH. Humanized anti-hepatocyte growth factor (HGF) antibody suppresses innate irinotecan (CPT-11) resistance induced by fibroblast-derived HGF. *Oncotarget* 2015; **6**: 24047–24060. [DOI: 10.18632/oncotarget.4369]

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Disoma C, Zhou Y, Li S, Peng J, Xia Z. Wnt/β-catenin signaling in colorectal cancer: Is therapeutic targeting even possible? *Biochimie* [Internet] 2022;**195**:39–53 [DOI: 10.1016/j.biochi.2022.01.009]Available from: https://linkinghub.elsevier.com/retrieve/pii/S0300908422000190

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Siveen KS, Raza A, Ahmed EI, Khan AQ, Prabhu KS, Kuttikrishnan S, Mateo JM, Zayed H, Rasul K, Azizi F, Dermime S, Steinhoff M, Uddin S. The Role of Extracellular Vesicles as Modulators of the Tumor Microenvironment, Metastasis and Drug Resistance in Colorectal Cancer. *Cancers (Basel)* [Internet] 2019;**11**:746 [DOI: 10.3390/cancers11060746]Available from: https://www.mdpi.com/2072-6694/11/6/746

Shao Y, Chen T, Zheng X, Yang S, Xu K, Chen X, Xu F, Wang L, Shen Y, Wang T, Zhang M, Hu W, Ye C, Yu X, Shao J, Zheng S. Colorectal cancer-derived small extracellular vesicles establish an inflammatory premetastatic niche in liver metastasis. *Carcinogenesis* [Internet] 2018;**39**:1368–79 [DOI: 10.1093/carcin/bgy115]Available from: https://academic.oup.com/carcin/article/39/11/1368/5090278

Abu N, Othman N, Ab Razak NS, Bakarurraini NAAR, Nasir SN, Soh JEC, Mazlan L, Azman ZAM, Jamal R. Extracellular Vesicles Derived From Colorectal Cancer Affects CD8 T Cells: An Analysis Based on Body Mass Index. *Front Cell Dev Biol* [Internet] 2020;**8** [DOI: 10.3389/fcell.2020.564648]Available from: https://www.frontiersin.org/articles/10.3389/fcell.2020.564648/full

Sameer AS, Nissar S. Toll-Like Receptors (TLRs): Structure, Functions, Signaling, and Role of Their Polymorphisms in Colorectal Cancer Susceptibility. *Biomed Res Int* [Internet] 2021;**2021**:1–14 [DOI: 10.1155/2021/1157023]Available from: https://www.hindawi.com/journals/bmri/2021/1157023/

Angrini M, Varthaman A, Cremer I. Toll-Like Receptors (TLRs) in the Tumor Microenvironment (TME): A Dragon-Like Weapon in a Non-fantasy Game of Thrones [Internet]. 2020. page 145–73Available from: http://link.springer.com/10.1007/978-3-030-44518-8_9

Kamal Y, Schmit SL, Frost HR, Amos CI. The tumor microenvironment of colorectal cancer metastases: opportunities in cancer immunotherapy. *Immunotherapy* [Internet]

2020;**12**:1083–100 [DOI: 10.2217/imt-2020-0026]Available from: https://www.futuremedicine.com/doi/10.2217/imt-2020-0026

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