# Response Letter to World Journal of Gastrointestinal Oncology

**Manuscript ID:** 89370

**Manuscript Title:** Expression and significance of pigment epithelium-derived factor and vascular endothelial growth factor in colorectal adenoma and cancer

We sincerely thank the reviewers and editors for their valuable comments, which helped us improve the quality of the manuscript. Comments from reviewers and editors are shown in italics below, and specific questions are numbered. Our responses are given in normal font and in blue text.

**1.Responses to Editor's Comments**

*Editor's Comment 1: The abbreviations do not exactly follow the rules of the sections.*

Response 1: Thank you for pointing out the irregularities in the abbreviations of this article. According to the basic rules of abbreviations you gave, we checked various parts of the article, found irregular positions of abbreviations in keywords, core prompts, charts, tables, and made modifications. Page 6 of the uploaded manuscript and figures have been revised and highlighted in yellow.

*Editor's Comment 2: Please provide the Figures cited in the original manuscript in the form of PPT. All text can be edited, including A,B, arrows, etc. With respect to the reference to the Figure, please verify if it is an original image created for the manuscript, if not, please provide the source of the picture and the proof that the Figure has been authorized by the previous publisher or copyright owner to allow it to be redistributed. All legends require a general title and explanation for each figure. Such as A: ; B: ; C: .*

Response 2:Thank you for your suggestions on the chart. We have downloaded your journal's guidelines for the preparation of bitmaps, vector graphics and tables in the revised manuscript, and we have revised the charts according to the guidelines and your editor's requirements. For details, please see the figures and tables in the uploaded manuscript. At the same time, we also provided the corresponding decompressible and editable graphs and

tables upon request, and sent the corresponding PowerPoint files and Word files to the designated destinations.

*Editor's Comment 3:The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text (and directly before the References).*

Response 3:Thank you for alerting us to missing parts of the manuscript. We have supplemented this section before the reference and marked it in yellow on pages 24 and 25 of the manuscript.

**2. Responses to Reviewer's Comment Comments**

*Reviewer's Comment 1:What are the new hypotheses that this study proposed?*

Response 1: Thank you for your questions about this manuscript. The hypothesis proposed in this study is that PEDF, as an antagonist of VEGF, may become a new target for early prevention and later treatment of CRC. The previous manuscript may not have been very compelling in terms of the hypotheses proposed. We have revised the manuscript and answered this question in the penultimate sentence of paragraph 2 on page 10 of the uploaded manuscript, highlighted in yellow.

*Reviewer's Comment 2:What are the new phenomena that were found through experiments in this study?*

Response 2: Thank you for your questions about this manuscript. We have discovered three new phenomena through this experiment. Firstly, the positive rate and intensity of PEDF expression in the adenoma group involved normal intestinal tissue and cancer tissue, which has rarely been investigated in previous studies. Secondly, PEDF expression intensity was negatively correlated with CD31-MVD value in both adenoma and colorectal groups, which is also a new research method and result in previous studies. (3) There was no significant correlation between the expression intensity of VEGF and the MVD value of CD31-MVD in colorectal adenoma. This is not consistent with the results of Wang Jinhui et al. The first two new findings are mentioned in the experimental results and in the discussion. As it has been

mentioned in the penultimate sentence of the first paragraph on page 10 of the manuscript that colorectal adenoma, a precancerous lesion, is rarely involved in the current research on PEDF at home and abroad, it is not further mentioned that these are new phenomena found in experiments. Regarding the third new finding, we explicitly state in the last paragraph on page 22 of the manuscript that this is an area of disagreement with the results of previous studies.

*Reviewer's Comment 3:What are the new concepts that this study proposes?*

Response 3:Thank you for your questions about this manuscript. Looking for a new target that can not only prevent colorectal adenoma from developing into colorectal cancer, but also treat colorectal cancer, this new concept deserves further research. Previous manuscripts may be less dramatic in presenting new concepts. The manuscript has been revised and elaborated on page 7, the first paragraph, and highlighted in yellow.

*Reviewer's Comment 4:What are the new methods that this study proposed?*

Response 4:Thank you for your questions about this manuscript. The aim of this study was to investigate the expression of PEDF and VEGF in normal colorectal mucosa, adenoma and colorectal cancer, and their relationship with clinicopathological features of colorectal cancer, starting from the earliest stages of colorectal cancer development, including colorectal adenoma, a precancerous lesion. At the same time, the microvessels were marked with CD31, and the MVD of each tissue was calculated to analyze the difference and correlation between the two. To investigate the role and significance of epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in the process of colorectal cancer from normal intestinal epithelium to adenoma and then to carcinoma. This is illustrated in the last paragraph on page 10 of the manuscript.

*Reviewer's Comment 5:What are the questions/issues that remain to be solved?*

Response 5:Thank you for your questions about this manuscript. In our experiment, the type of adenoma in the adenoma group was dominated by tubular adenoma, and the proportion of adenomas with various histological features was not balanced in the adenoma group. In addition, our overall sample size was not large, so it is necessary to further expand the sample size and balance adenomas with different histological features to further clarify and confirm our views. This is detailed in the first paragraph on page 23 of the manuscript.

*Reviewer's Comment 6: I ask the Editors to check whether the Authors sent the consent they received from the patient who participated in the study.*

Response 6:Thank you for your careful examination of this manuscript. We have resubmitted the ethically relevant materials including the informed consent form.

*Reviewer's Comment 7: I ask the Authors to note that colorectal cancer is the third most common cancer occurring worldwide with an estimated one million new cases diagnosed each year.*

Response 7: Thank you for your additional comments on the manuscript. It is explained in the last paragraph on page 5 of the paper and marked in yellow.

*Reviewer's Comment 8: Emphasize that in recent years, advances in the understanding of tumor biology have led to the development of targeted therapies that have provided advances in the treatment of colorectal cancer.*

Response 8: Thank you for your pertinent comments on this manuscript. We emphasize this point in the penultimate sentence of the first paragraph on the page of the manuscript.

*Reviewer's Comment 9:Explain that colorectal cancer is one of the many malignancies in which angiogenesis is involved. Discuss that angiogenesis is the formation of new capillaries either from endothelial progenitor cells or from pre–existing vasculature. It is important in different stages of malignant disease.*

Response 9: Thank you for your valuable comments on this article. The above comments are discussed and highlighted on page 7, paragraph 2, lines 2-4,

and the penultimate paragraph, sentences 2-4, on page 19 of the manuscript and are marked in yellow.

*Reviewer's Comment 10:Point out that VEGF is an important angiogenic factor in primary and metastatic human colorectal cancer.*

Response 10:Thank you for your valuable comments on this article. The above comments are highlighted in lines 3-4 on page 8 of the manuscript.

*Reviewer's Comment 11:Note that VEGF expression is correlated with an increased number of microvascular tumors in colon tumors and it is associated with poor outcomes as measured by tumor progression, metastases and patient survival.*

Response 11:Thank you for your valuable comments on this article. Our comments are elaborated on lines 1-6 from the bottom of page 7 and the first sentence on page 8 of the manuscript.

*Reviewer's Comment 12: Discuss that VEGF is a highly specific mitogen for vascular endothelial cells. Emphasize that Napoleon Ferrara discovered it in 1989 and in 1993 found that inhibition of VEGF–induced angiogenesis with specific monoclonal antibodies resulted in dramatic suppression of the growth of various tumors in vivo. These findings provided important evidence that inhibition of angiogenesis can suppress growth and lead to tumor blocking.*

Response 12:Thank you for your valuable comments on this article. The above comments are discussed in the last paragraph on page 7 of the paper and are marked in yellow.

*Reviewer's Comment 13: Point out that VEGF and VEGFR also regulate vasculogenesis (the development of blood vessels from precursor cells during early embryogenesis) and angiogenesis from already existing blood vessels at a later stage.*

Response 13:Thank you for your valuable comments on this article. The above comments have been discussed in the second sentence of the second paragraph of the paper on page 21 and are marked in yellow.

*Reviewer's Comment 14:Explain that scientific studies have found that VEGF is expressed early in the progression of colorectal cancer and the VEGF expression is correlated with increased microvessels in colon tumors and is associated with poor*

*outcomes as measured by tumor progression, metastases and patient survival.*

Response 14:Thank you for your valuable comments on this article. Our comments are elaborated in the lines 1-6 at the bottom of page 7 of the manuscript and in the first sentence on page 8.

*Reviewer's Comment 15:Discuss that in addition to evidence suggesting that VEGF is a prognostic factor in colorectal cancer, studies have shown that VEGF can predict response to conventional systemic therapy and local radiotherapy.*

Response 15: Thank you for your valuable comments on this article. These comments have been discussed on page 8 of the manuscript, lines 5-11, and are highlighted in yellow.

*Reviewer's Comment 16:Explain that pigment epithelium–derived factor (PEDF) also known as early population double level c DNA–1 (EPC–1) is a 50 kDa secreted glycoprotein. Point out that it was first identified when Tromban–Tink's group studied the development of human retinal cells. Emphasize that they found a factor secreted by human fetal retinal pigment epithelial cells and showed that it is a potent neurite-promoting factor.*

Response 16:Thank you for your valuable comments on this article. We have mentioned some of the above opinions in the original manuscript, and we have also made further supplements according to your suggestions. It is elaborated and supplemented in the last paragraph on page 8 and the first paragraph on page 9 of the manuscript.

*Reviewer's Comment 17:Note that there is evidence that PEDF is pleiotropic with multiple biological properties including neuroprotective, antitumorigenic and immunomodulatory effects and has been shown that it is one of the most potent endogenous inhibitors of angiogenesis, more than angiostatin, endostatin and trombospondin–1.*

Response 17:Thank you for your valuable comments on this article. We elaborate in lines 5-6 on page 9 of the manuscript and in the penultimate sentence of paragraph 1 on page 9.

*Reviewer's Comment 18:Explain that PEDF exerts anti–angiogenic activities by inhibiting endothelial cell proliferation and migration, an activity that has been shown to occur even in the presence of vascular endothelial growth factor (VEGF).*

Response 18:Thank you for your valuable comments on this article. We have provided explanations on page 22 of the manuscript, highlighted in yellow.

*Reviewer's Comment 19:Emphasize that the proposed underlying mechanisms of the biological effects of PEDF on endothelial cells involve the complex cross–talk between signaling events triggered by both proangiogenic and anti–angiogenic molecules.*

Response 19: Thank you for your valuable comments on this article. We highlight in yellow lines 10-12 on page 22 of the manuscript.

*Reviewer's Comment 20:Discuss that PEDF expression is lower in solid tumor tissue compared to normal tissue from the same organ, suggesting that loss of PEDF expression may play a key role in tumorigenesis.*

Response 20:Thank you for your valuable comments on this article. We discuss this in the penultimate paragraph on page 9 of the manuscript.

*Reviewer's Comment 21:Point out that PEDF expression is decreased with worsening prognostic factors in a range of cancers and that treatment with recombinant PEDF has shown some benefit in cellular functional models, possibly in part due to PEDF inhibiting aberrant angiogenesis, leading to normalization of healthy vascularization.*

Response 21:Thank you for your valuable comments on this article. We have described them on page 8 of the manuscript, lines 9-10 and 13-15, and highlighted them in yellow.

*Reviewer's Comment 22:Emphasize that pigment epithelium–derived factor (PEDF) is called serine protease inhibitor F1 (SERPINF1). Note that the most important function of PEDF is its ability to inhibit angiogenesis, the process that drives tumor growth and metastases.*

Response 22:Thank you for your valuable comments on this article. We have highlighted this on page 9, lines 3-6, 10.

*Reviewer's Comment 23:Explain that in various in vivo models, PEDF reduced microvascular density in tumor tissues, which is one of the mechanisms leading to*

*tumor inhibition.*

Response 23:Thank you for your valuable comments on this article. The above recommendations are outlined on page 9, paragraph 2, lines 5 through 7 of the manuscript.

*Reviewer's Comment 24:Emphasize that in in vitro assays PEDF's anti–angiogenic activity is more potent than other endogenous anti–angiogenic factors such as endostatin, angiostatin and thrombosponding–1.*

Response 24:Thank you for your valuable comments on this article. We have addressed this in lines 4-6 on page 9 of the manuscript.

*Reviewer's Comment 25:Discuss that the PEDF receptor mediates the antiangiogenic activity of pigment epithelium–derived factor and has been identified as a transmembrane cell surface protein containing a plexin domain (PLXDC1), which is also known as tumor endothelial marker 7 (TEM 7).*

Response 25:Thank you for your valuable comments on this article. Our discussion of the above recommendations is discussed and highlighted in yellow on page 20 of the manuscript.

*Reviewer's Comment 26:Point out that PLXDC1 and its homolog PLXDC2 are the only two proteins that have been shown to bind the extracellular PEDF to the cell surface and to transduce the PEDF signal into the cell.*

Response 26:Thank you for your valuable comments on this article. Our recommendations for the above are highlighted and marked in yellow on page 20 of the manuscript, lines 11 through 13.

*Reviewer's Comment 27: Lead a discussion that plexin domain containing 1 (PLXDC1) / tumor endothelial marker (TEM7) was highly enriched in blood vessels of tumor tissues, but not in blood vessels of adjacent normal tissue.*

Response 27:Thank you for your valuable comments on this article. Our discussion of the above recommendations is highlighted in yellow on page 20 of the manuscript.

*Reviewer's Comment 28:Explain that PLXDC1 receptor expression has been found in a wide variety of cancer types including liver cancer, breast cancer, ovarian cancer,*

*pancreatic cancer, colorectal cancer, lung cancer, neuroblastoma and sarcomas.*

Response 28:Thank you for your valuable comments on this article. Our recommendations are outlined in lines 9 through 11 from the bottom of page 20 of the manuscript.

*Reviewer's Comment 29:Emphasize that this high specificity of PEDF receptor expression in tumor blood vessels corresponds to the specificity of PEDF's tumor inhibitory effect. Note that in addition to inhibiting angiogenesis, PEDF also directly inhibits certain tumor cells and promotes cell differentiation.*

Response 29:Thank you for your valuable comments on this article. Our recommendations for the above are highlighted on the penultimate lines of page 9 of the manuscript.

*Reviewer's Comment 30:Point out that PEDF has been found to induce tumor cell differentation of neuroblastoma tumor cells and promote neuroendocrine function in prostate cancer cells.*

Response 30:Thank you for your valuable comments on this article. Our above recommendations are illustrated on page 9, lines 8-10.

*Reviewer's Comment 31:Explain that another PEDF receptor, PLXDC2 is expressed in a variety of cancers including colon cancer, hepatocellular cancer, laryngeal cancer, testicular seminoma and squamous cell cancer of the vulva.*

Response 31:Thank you for your valuable comments on this article. An explanation of our recommendations is provided on page 20 of the manuscript, lines 1-4.

*Reviewer's Comment 32:Emphasize that signaling mediated by PLXDC2 receptor is responsible for the direct effect of PEDF on cancer cells. Note that collective anti–cancer activity (anti–angiogenesis and direct effect of antitumor cells) of PEDF has been observed in in vivo studies.*

Response 32:Thank you for your valuable comments on this article. Our recommendations for the above are marked in yellow on page 21, paragraph 1, of the manuscript.

*Reviewer's Comment 33:Point out that PEDF treatment with gene therapy or*

*recombinant protein has been proven to inhibit the growth of pancreatic cancer, hepatoblastoma, prostate cancer, retinoblastoma, ocular melanoma, lung cancer and colon cancer. Emphasize that PEDF significantly reduced thoracic metastases of colon cancer.*

Response 33:Thank you for your valuable comments on this article. Our recommendations are emphasized in the penultimate paragraph on page 9 of the manuscript.

*Reviewer's Comment 34:Discuss that PEDF is widely expressed in most human organs and tissues such as the eye, liver, heart, brain, bones and lungs.*

Response 34:Thank you for your valuable comments on this article. Our recommendations for the above are emphasized 6 to 10 lines from the bottom of the first paragraph on page 9 of the manuscript.

*Reviewer's Comment 35:Note that significant reductions in PEDF levels have been found in age–related macular degeneration and diabetic retinopathy, two pathological processes dependent on angiogenesis.*

Response 35:Thank you for your valuable comments on this article. Our above suggestions are highlighted in lines 7-8 on page 9.

*Reviewer's Comment 36:Discuss that PEDF exerts a variety of biological effects in many physiological and pathophysiological processes including neuroprotection, fibrogenesis, and inflammation.*

Response 36:Thank you for your valuable comments on this article. These comments are addressed in the last sentence of the first paragraph on page 9 of the manuscript.

*Reviewer's Comment 37:Point out the importance of the fact that PEDF has numerous biological functions, including differentiating activity, neurite outgrowth, survival activity, anti–apoptosis and antiangiogenic activities and induction of cell death.*

Response 37:Thank you for your valuable comments on this article. For the above comments, we highlight them in lines 5-7 of the last paragraph on page 9 of the manuscript.

*Reviewer's Comment 38:Note that PEDF can induce tumor differentiation into a less*

*malignant phenotype and can block angiogenesis-mediated activities and neovascularization.*

Response 38:Thank you for your valuable comments on this article. We elaborated in the last sentence on page 21 and the first paragraph on page 22 of the manuscript and marked them in yellow.

*Reviewer's Comment 39:Note that PEDF can suppress tumor cell invasion and metastasis and exhibits anti-angiogenic effects in various tumor models including retinoblastoma, neuroblastoma, prostate cancer, melanoma, Wilms tumor, pancreatic adenocarcinoma, hepatoblastoma, osteosarcoma, chondrosarcoma, human cervical carcinoma, gastric carcinoma, nasopharyngeal carcinoma, colorectal peritoneal carcinoma, glioma and breast carcinoma xenografts.*

Response 39:Thank you for your valuable comments on this article. Our recommendations regarding the above are emphasized on page 9 of the manuscript, in the second paragraph, lines 5 through 7.

*Reviewer's Comment 40:Discuss that the antiangiogenic effect of PEDF is achieved primarily through disruption of the distribution of the microvascular network. Vascular endothelial growth factor (VEGF) expression is an established proangiogenic factor and numerous studies have reported about the inverse correlation between PEDF and VEGF expression levels in certain tumor models.*

Response 40:Thank you for your valuable comments on this article. The above comments are highlighted in yellow and discussed on page 22 of the manuscript.

*Reviewer's Comment 41:Cite this manuscripts:*
*Balasubramanian S, Priyathersini N, Johnson T. Expression of Vascular Endothelial Growth Factor (VEGF) in Colorectal Adenoma and Carcinoma in a Tertiary Care Center. Cureus. 2022 Nov 11;14(11):e31393. doi: 10.7759/cureus.31393. PMID: 36514651; PMCID: PMC9742501.*

Response 41:Thank you for your valuable comments on this article. This reference is a more recent one relevant to this paper, and we cite it in reference 15.

*Reviewer's Comment 42:Cite this manuscripts:*

*Wang Y, Liu X, Quan X, et al. Pigment epithelium-derived factor and its role in microvascular–related diseases. Biochimie. 2022; 200:153–171. doi: 10.1016/j.biochi.2022.05.019*

Response 42:Thank you for your valuable comments on this article. This reference is a more recent one relevant to this paper, and we cite it in reference 12.