

Dear Editor:

We wish to thank the editor and the reviewer for their comments and would like to resubmit the revised version of our manuscript entitled “EFNA1 in gastrointestinal cancer: expression, regulation and clinical significance” (Manuscript NO: 67296). We have revised the manuscript according to the comments of the reviewers, and highlighted the changes in red in the revised version of the manuscript. A detailed description of the changes made is provided in the Response to the reviewers following this letter.

All authors have read and approved this revised version of the manuscript. No part of this paper has been published or submitted elsewhere, and no conflict of interest exists for any of the authors. We appreciate your reconsideration of our manuscript.

Sincerely,

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Response to the reviewers

(Changes are highlighted in red in the revised version of the manuscript)

Reviewer #1: Scientific Quality: Grade C (Good); Language Quality: Grade B (Minor language polishing); Conclusion: Minor revision

Comment 1: The manuscript is well written but the abstract and introduction can be summarized.

Response: Thanks for your suggestion. We summarized the abstract and introduction, which are highlighted in red in the revised manuscript. (page 2,4 of the revised manuscript)

Reviewer #2: Scientific Quality: Grade B (Very good); Language Quality: Grade B (Minor language polishing); Conclusion: Major revision

Dear Authors, A very comprehensive review with special attention on molecular aspects (great work with possible mechanisms of angiogenesis and therapeutic aspects or perspective for the future). Overall, the article can be recommended but not without proper revisions. My suggestions/concerns/questions are below:

Comment 1: Abstract, the second sentence. It is quite long and please consider rephrasing it a little. What I also suggest is to change “and” words to something else since there is quite a few of them. For example: “The ephrins and EPH-related receptors comprise the largest subfamily of receptor protein-tyrosine kinases WHICH play an indispensable role in normal growth and development OR in the pathophysiology of various tumors”

Response: Thanks for your suggestion. According to your suggestion, we revised this sentence, which now reads “The ephrins and EPH-related receptors comprise the largest subfamily of receptor protein-tyrosine kinases WHICH play an indispensable role in normal growth and development OR in the pathophysiology of various tumors” (page 2 of the revised manuscript)

Comment 2: Introduction, all sentences up to first (“[1]”) reference. Are all these data from one citation?

Response: It is very nice of you to review our paper carefully. And all sentences up to first (“^[1]”) reference from one citation.

Comment 3: Introduction, sentence “Studies have shown that the EphA2 receptor and its ligand ephrin-A1 are expressed in a variety of malignant tumors, and the interaction between the two promotes the migration of tumor vascular endothelial cells [4]”. I suggest changing the last “the” word to “these”.

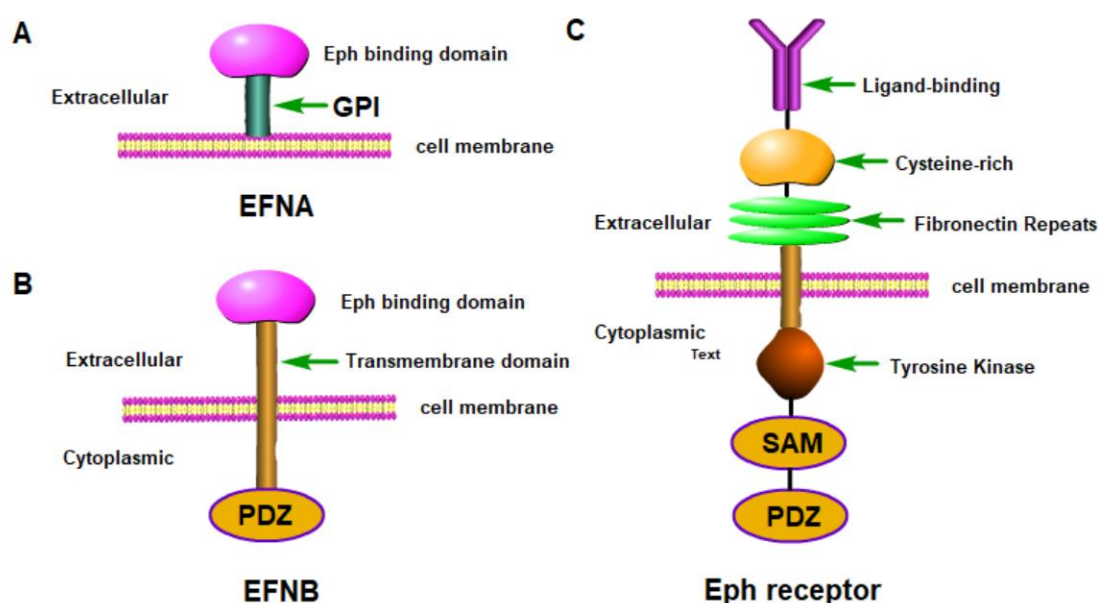
Response: Thanks for your suggestion. We have changed the last “the” word to “these”, which now reads “Studies have shown that the EphA2 receptor and its ligand ephrin-A1 are expressed in a variety of malignant tumors, and the interaction between the two promotes THESE migration of tumor vascular endothelial cells ^[4]” (page 4 of the revised manuscript)

Comment 4: Add hyphen in “EphrinB” (page 5), since you use it for all other cases (e.g. Ephrin-A).

Response: We sincerely appreciate your comment. And have added hyphen in “EphrinB”, which now reads “Ephrin-B” (page 5 of the revised manuscript)

Comment 5: Figure 1. I suggest to split subfigure A into subfigure A and B to underline changes between EFNA and EFNB (at first glance I was unable to spot it). The current subfigure B would be subfigure C in that case.

Response: Thanks for your suggestion. We have modified Figure 1 according to your requirements.



Comment 6: The last sentence on page 6. I would add “through” or “by” after “activated” in “When EphA2 is activated ephrin-A1 binding, (...)”.

Response: It is very nice of you to review our paper carefully. We have revised this sentence, which now reads “When EphA2 is activated THROUGH ephrin-A1 binding,ultimately resulting in the signal transduction complex.” (page 6 of the revised manuscript)

Comment 7: Table 2. Is the symbol “(-)” represents lack of data?

Response: Yes, the symbol “(-)” represents lack of data.

Comment 8: Third sentence in “Gastric cancer” section. The end of this sentence is hard to understand. If I am not mistaken, will this version work? “EFNA1 is highly expressed in GC tissues, but is low or not expressed in benign GC lesion; its expression increases with malignant status.”

Response: We sincerely appreciate your comment. We have changed the last “but” word to “and”, which now reads “EFNA1 is highly expressed in GC tissues,AND its expression increases with increases in malignancy^[30]” (page 7 of the revised manuscript)

Comment 9: The sentence starting with “Increases in EFNA1 expression parallel increases” is hard to understand, please rewrite it. I do have suggestion for the beginning of this sentence e.g. “EFNA expression increases with both clinical stage and lymph node metastasis (...)”.

Response: It is very nice of you to review our paper carefully. According to your suggestion, we revised this sentence, which now reads “EFNA1 expression increases with both clinical stage and lymph node metastasis, and decreases in the degree of tissue differentiation, which indicates the malignant degree of GC” (page 7 of the revised manuscript)

Comment 10: The last sentence in “Gastric cancer” section. The part after the last comma would be better if it sounds like “(...) and metastasis of GC cells; up-regulation of GMAN is also associated with poor prognosis of GC.”

Response: Thanks for your suggestion. We have revised this sentence, which now reads “GMAN regulate.....and metastasis of GC cells; up-regulation of GMAN is associated with poor prognosis of GC^[37].” (page 8 of the revised manuscript)

Comment 11: This sentence is hard to understand, please rewrite it. “Among them, from adenoma to cancer tissue, the copy number and mRNA expression of EFNA1 increased, indicating that EFNA1 may be a driver gene that promotes rectal adenocarcinoma-rectal cancer”.

Response: We sincerely appreciate your comment. We have rewritten this sentence, which now reads “Among them, the gene copy number and mRNA expression of EFNA1 increased in the progression from adenoma to cancer, indicating that EFNA1 may be a driving gene to promote rectal cancer.” (page 9 of the revised manuscript)

Comment 12: The sentence starting with “A study by Rosenberg et al. showed that the CRC epithelial cell line Caco-2” is very long. Consider splitting it to two separate sentences or add semicolon somewhere.

Response: Thanks for your suggestion. We have divided it to two separate sentences, which now reads “A study by Rosenberg et al. its receptor EphA2 (Eck). The ephrin-A1 and EphA2 the epithelial barrier^[46].” (page 9 of the revised manuscript)

Comment 13: The sentence starting with “Kataoka et al. detected the expression of EFNA1 in CRC specimens and found that 62.5% (25/37)”. I suggest changing the second part of it to “62.5% (25/37) expressed ephrin-A1 in greater extend which correlated with low survival rate and poor prognosis”

Response: It is very nice of you to review our paper carefully. We have revised this sentence, which now reads “Kataoka et al. detected the expression of EFNA1 in CRC specimens and found that 62.5% (25/37) expressed ephrin-A1 in greater extend which correlated with low survival rate and poor prognosis” (page 9 of the revised manuscript)

Comment 14: The last sentence in “Colorectal cancer” section. Just my curiosity, but are you able to perform such multi-center clinical study in the future?

Response: We sincerely appreciate your comment. At present, we have cooperated with the First Affiliated Hospital of Shantou University Medical College and Sun Yatsen University Cancer Center. In addition, we are actively seeking cooperation with other hospitals, so we believe that multi-center research can be carried out in the near future.

Comment 15: Citation “[57,58]” on page 10 is not upper indexed.

Response: Thanks for your suggestion. We have changed the citation “[57,58]” to “[57,58]”.

Comment 16: The sentence starting with “A study by Lida et al. [57] showed that ephrin-A1”. In terms of cell cycle, are there any other proteins than p21 which were regulated by ephrin-A1?

Response: Thanks for your suggestion. Through literature search, we found that in addition to p21, other proteins such as CyclinD1 and pLE are also regulated by ephrin-A1. However, most of these studies are in the endometrium, so we did not add this part to the section of Hepatocellular carcinoma.

Comment 17: Section “Esophageal cancer”. Abbreviation “ESCC” is explained in the third sentence while it should be in the second one.

Response: Thanks for your suggestion. We have explained the abbreviation "ESCC" in the second sentence. (page 11 of the revised manuscript)

Comment 18: The sentence starting with “The results showed that 84.4% (146/173) positively expressed”. Are the values in brackets represents number of tissue samples? If so, please add “sample” or “specimen” somewhere in this sentence, as this could be not clear for all readers at first glance.

Response: It is very nice of you to review our paper carefully. And the values in brackets represents number of tissue samples. We have revised this sentence, which now reads “The results showed that 84.4% (146/173) sample positively expressed, and 15.6% (27/173) sample negatively expressed EFNA1.” (page 11 of the revised manuscript)

Comment 19: The sentence starting with “Univariate analysis showed that a high level of EFNA1 protein expression”. Is the high level of EFNA1 also independently associated with metastasis, grade or stage? Or this is only with OS?

Response: Thanks for your suggestion. We have revised this sentence, which now reads “In addition to overall survival, EFNA1 protein expressions were significantly associated with histological grade, number of lymph node metastasis and clinical stage, for patients with ESCC in the univariate analysis ^[59].” (page 11 of the revised manuscript)

Comment 20: The sentence starting with “In addition, studies have also shown that ephrin-A1 and EphA2 often co-localize”. I feel something might be missing in this sentence, or the ending “related to co-localize” should be “related to co-localization”.

Response: We sincerely appreciate your comment. We have revised the end of the sentence, which now reads “In addition, studies have also shown that ephrin-A1 and EphA2 often co-localize in the tumor area and vascular endothelial cells in ESCC, and their expression is related to co-localization ^[59].” (page 11 of the revised manuscript)

Comment 21: The first reference in “Role of EFNA1 in gastrointestinal cancers” section. Is the first sentence of this section really summarized citations from no. 28 to 61?

Response: It is very nice of you to review our paper carefully. Yes, the first sentence of this section summarized citations from no. 28 to 61.

Comment 22: Change “a” to “an” before “increase” in sentence “Potla et al. [42]

found that in three- dimensional spheroid cultures of HT29 colon cancer cells, a increase of EFNA1”.

Response: Thanks for your suggestion. We have changed the “a” word to “an”, which now reads “Potla et al. ^[42] found that in three- dimensional spheroid cultures of HT29 colon cancer cells, an increase of EFNA1 expression reduces the growth of tumor cells.” (page 12 of the revised manuscript)

Comment 23: The last sentence of “Regulation of gastrointestinal cancer cell growth” could be rewritten, especially the end of it. I suggest “its abnormal expression in cancers can affect tumor growth and formation”.

Response: Thanks for your suggestion. We have rewritten this sentence, which now reads “Therefore, in a sense, EFNA1 can be considered as a potential growth factor ^[65], and its abnormal expression in cancers can affect tumor growth and formation” (page 12 of the revised manuscript)

Comment 24: Sentence starting with “In addition, studies have shown that the density of ephrin-A1”. What do you mean by “density” here? Maybe the “amount” would fit better?

Response: We sincerely appreciate your comment. We have changed the “density” word to “amount”, which now reads “In addition, studies have shown that the amount of ephrin-A1 determines the extent of EphA2-dependent, integrin-mediated cell adhesion ^[68].” (page 13 of the revised manuscript)

Comment 25: To the entire manuscript: if you use EFNA1 for gene, remember to use italics. Please double-check if you have it properly done. Also, “in vitro” and “in vivo” should also be italicized.

Response: Thanks for your suggestion. We have shown “EFNA1”, “in vitro” and “in vivo” in italics.

Comment 26: RT-PCR abbreviation was explained on page 13 while it should be at least on page 11.

Response: It is very nice of you to review our paper carefully. We have explained the abbreviation “ RT-PCR ” on page 11.

Comment 27: First sentence in “EFNA1 AND TUMOR ANGIOGENESIS” section. “tumor process” could be changed to “carcinogenesis” to avoid using triple “tumor” in the same sentence.

Response: Thanks for your suggestion. We have changed the “tumor process” word to

“carcinogenesis” , which now reads “Tumor angiogenesis is a common pathological phenomenon in the carcinogenesis, and directly regulates the pathological process of tumor growth, invasion, and metastasis.” (page 14 of the revised manuscript)

Comment 28: Sentence with “new blood vessels can be used as a metastasis channel”. I think there should be “metastasis channelS” since you have “blood vesselS”. Also, I would go for “metastatic channels” to avoid “metastasis” repetition.

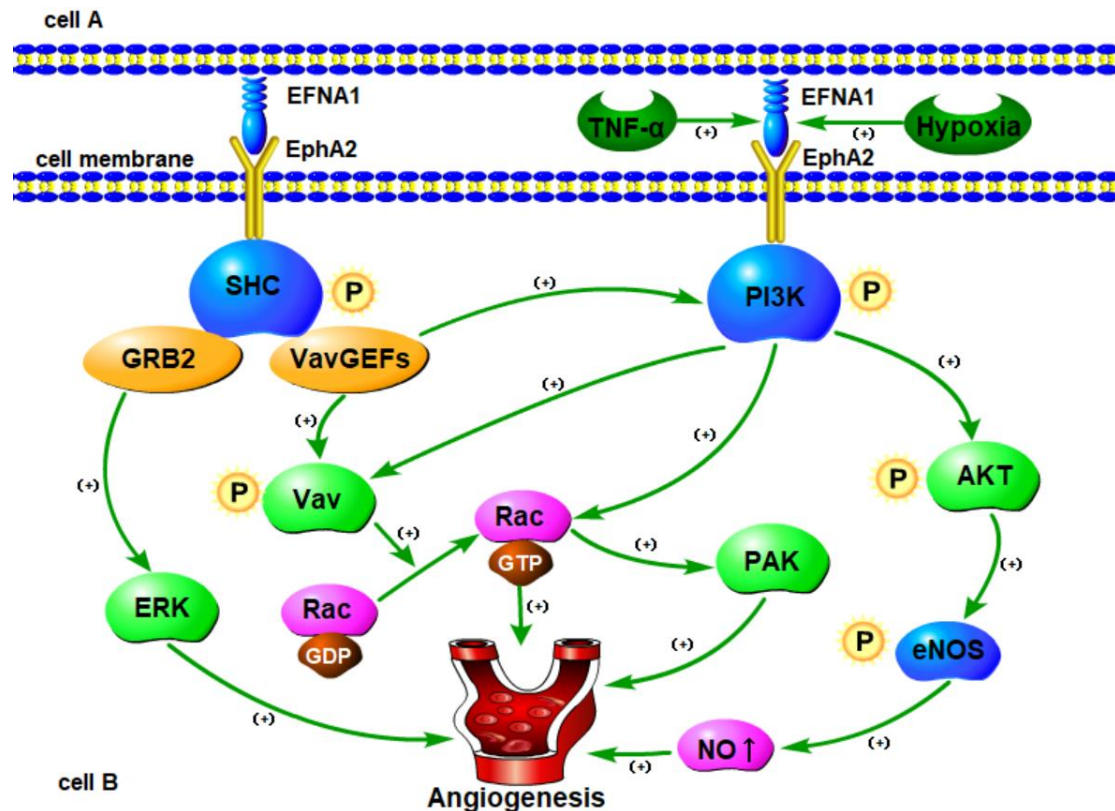
Response: Thanks for your suggestion. We have changed the “channel” word to “channels” , which now reads “At the same time, new blood vessels can be used as a metastasis channels to mediate distant metastasis of tumors ^[82].” (page 14 of the revised manuscript)

Comment 29: Sentence starting with “In 2000, Ogawa et al”. I would add “that” after “showing”.

Response: It is very nice of you to review our paper carefully. We have added “that” after “showing”, which now reads “In 2000, Ogawa et al. showing that overexpressionreduces microvascular density ^[84].” (page 15 of the revised manuscript)

Comment 30: Figure 3. Change “cell membrane” to “cell membrane”. Also, I would standardize the size of lipid bilayer for both cells and add some text to clearly indicate that we have one bilayer from one cell and second from another.

Response: Thanks for your suggestion. We have modified Figure 3 according to your requirements.



Comment 31: Table 1, column “Encoding mRNA and protein”. Delete space between comma and “NP_004419.2”.

Response: Thanks for your suggestion. We have deleted the space between comma and “NP_004419.2”.

Comment 32: Could you add some information about one of gastrointestinal cancers which is pancreatic cancer? I think making the subsection in the main section “EFNA1 AND GASTROINTESTINAL CANCERS” would do the job.

Response: We sincerely appreciate your comment. Pancreatic cancer is also a gastrointestinal cancer. Therefore, we first considered the relationship between EFNA1 and pancreatic cancer, but no literature was reported. According to your suggestion, we searched again, but also no relevant literature was found. In the revised manuscript, we added this in the Conclusion and perspectives section. Specifically, we now state “However, the research on EFNA1 and pancreatic cancer is still In addition, the specific molecular mechanism of EFNA1 in tumor progression is still poorly understood, and many aspects remain to be explored.” (page 22 of the revised manuscript)