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**Dear Science Editor Yuan Qi and reviewers,**

Thank you for your positive comments and detailed instructions for further revision of our manuscript *"Pleiotrophin and N-syndecan promote perineural invasion and tumor progression in an in situ pancreatic cancer mouse model"*. I have done the requested revisions. Here are the responses for the referees.

Your sincerely
JUN YAO
2016-10-22

**Reviewer's code:** 03086186

**Response:**

1. According to your proposal, we have revised the conclusion: High PTN and N-syndecan expression was closely associated with metastases and poor prognosis, suggesting that they may promote tumor progression and PNI in an in situ mouse model of pancreatic cancer.

2. In the study, PTN and N-syndecan expression were evaluated by independent observers, rather than using the software to quantify the intensity of protein expression. The main purpose was that ensure PTN and N-syndecan expression were consistent. In previous similar clinical study, we also investigated pleiotrophin (PTN) and N-syndecan protein levels in 38 patients with pancreatic cancer by two independent observers.

**Reviewer's code:** 03087223

**Response:**

1. We have used the primary antibodies of N-syndecan that made by Santa Cruz Biotechnology, but the staining seems was the same.

2. Because the N-Syndecan expression was originally less in the perineurium of pancreatic cancer, so, the staining seems very weak.

3. Peptide competition assay is more expensive, in the subsequent experiments we strive to use this peptide competition assay to confirm the specificity of the staining.

4. In the study, the results showed that mouse tissue expression of PTN and N-syndecan proteins were 66.7% and 61.1%, respectively. PTN and N-syndecan expressions were associated with PNI. But the N-syndecan expression was not significantly associated with survival time, the reason we think was less sample size, only 36 mice who survived for more than 90 days.

5. We have proofread this sentence: "Pleiotrophin (PTN) is a type of neurotrophic factor, also known as neurite growth-promoting factor, found in human, mouse, and rat".

**Reviewer's code:** 03087211

**Response:**

1. English has been improved throughout the manuscript by a native English professional with science background at Scientific Writing Solutions Corporation, USA.

2. Quantitative information has been provided in the abstract.

3. The members in the experiment think that the contents with the citation of the references you provided are inappropriate in this article. In the subsequent articles about the study, we strive to use these references.

**Reviewer's code:** 03089133

**Response:**

1. In our previous experiments in vitro, the expression of PTN in MiaPaCa-2 line is more compelling.

2. Gender was considered as an influential factor in the study, we selected male nude mice as the research object, and the reason was only ensure the consistency between the objects of research.

3. The results of PTN staining in figure 1 were evaluated in terms of the percentage of positive-stained cells by two independent observers, with 20~30% considered moderate PTN expression and ＞30%

considered intense PTN expression.

4. Because the N-Syndecan expression was originally less in the perineurium of pancreatic cancer, so, the difference of N-syndecan staining is not obvious between figure 1C and figure 1D.

5. Related experiment in vivo about the study has not been performed previously, in the subsequent experiments, we will complete some mechanistic studies about pleiotrophin and N-syndecan promote perineural invasion and tumor progression.

**Reviewer's code:** 03062291

**Response:**

1. The study was an experiment in vivo, not been performed previously. The results were consistent with the results of original research; the study does not provide enough novelty. In the subsequent experiments, we will complete some mechanistic studies about pleiotrophin and N-syndecan promote perineural invasion and tumor progression

2. The expression of PTN correlates with the expression of N-syndecan can hardly explain the difference in survival rates in Fig 2, the reason we think was less sample size, only 36 mice who survived for more than 90 days.

3. The hybridization in situ method would be good to test the expression of PTN and N-syndecan. In the subsequent experiments, we will complete some mechanistic studies using the hybridization in situ method.

4. Gender was considered as an influential factor in the study, we selected male nude mice as the research object, the reason was only ensure the consistency between the objects of research.