Peer Review File

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Review Comments

Comment 1: The authors have not shown the prognosis of patients between high and low expressions of EphB3. It is critical information in this study.

Reply 1: Follow-up data of these patients were collected and statistically analyzed. We found patients with high EphB3 expression had poorer prognosis in 3-year overall survival (OS). The results were shown in detail in Figure 2 and the new Table 3.

Changes in the text: We have added some data to the abstract (see Line 34-35), materials and methods (see Line 191-192), result (see Line 197-198, 211-216) and discussion section (see Line 267). We added a result in Figure 2 and modified the figure legend (see Line 401-405). We also added a new table (Line 462-465).

Comment 2: EphB3 is a member of the tyrosine kinase receptor. Therefore, it is reasonable that EphB3 knockdown downregulated pAKT. In contrast, the mechanism of how EphB3 influenced the expressions of EMT-related proteins. Would you please demonstrate the mechanism?

Reply 2: Since EMT was looked on as a critical downstream effect of the AKT signaling pathway, we used AKT activator SC79 to observe whether AKT pathway was involved in EphB3-regulated expressions of EMT-related proteins. We found that elevated AKT phosphorylation level could block the suppressive effects of EphB3 knockdown on EMT, as well as those on cell proliferation, migration and invasion. The results were shown in the new Figure 6.

Changes in the text: We have added some data to the abstract (see Line 38-42), materials and methods (see Line 110-111), result (see Line 242, 246-250) and discussion section (see Line 295-300). we added a figure legend to Figure 6 (see Line 431-437)

Comment 3: Previous studies demonstrated that EphB3 suppressed cell proliferation and invasion in the other types of tumors. Why does this molecule act differentially among the cell types? Could you provide the reason?

Reply 3: The Eph receptors and their ephrin ligands have intriguing expression patterns in different type of cancers. Unlike prototypical RTK, Eph-ephrin complexes emanate bidirectional signals: forward signals dependent on Eph kinase activity are often found in the receptor-expressing cell and reverse signals dependent on Src family kinases propagate in the ephrin-expressing cell. Kinase independent Eph signals can also occur. Eph signaling activities in cancer appear to be complex and their roles can be either tumor suppressive or oncogenic based on the cancer type and the cellular context. The Eph receptors represent promising new therapeutic targets in cancer. It is important to clarify the dichotomy of Eph receptors and how their dysregulated expression can promote or inhibit tumor progression.

EphB3 has been reported as a key player in multiple cancers. It exerted tumor suppressive role in human colorectal cancer and gastric cancer (1, 2). However, in non-small cell lung cancer (NSCLC), EphB3 acted as a tumor promoter in a kinase-independent manner (3), and forced activation of EphB3 kinase appeared inhibitory effect (4). EphB3 was also hypo-phosphorylated in head and neck squamous cell cancer, and forced EphB3 activation with an ephrin-B2-Fc fusion protein resulted in decreased cell growth and migration (5). This perplexing dichotomy was attributed to differences in kinase activation status of the EphB3 receptor.

In present study, we investigated the expression profile and role of EphB3 in esophageal carcinoma. Next, we would further investigate whether the role of EphB3 is kinase-dependent or not and identify the effects of various phosphorylation levle of EphB3 on tumor progression and the potential mechanism.

Changes in the text: We added some content in introduction. (see Line 75-77)

References

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progression. Nature 2005;435(7045):1126-30.

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3. Ji XD, Li G, Feng YX, et al. EphB3 is overexpressed in non-small-cell lung cancer and promotes tumor metastasis by enhancing cell survival and migration. Cancer Res 2011;71(3):1156–1166.
4. Li G, Ji XD, Gao H, et al. EphB3 suppresses non-small-cell lung cancer metastasis via a PP2A/RACK1/Akt signalling complex. Nat Commun 2012;3:667.
5. Bhatia S, Griego A, Lennon S, et al. Role of EphB3 Receptor in Mediating Head and Neck Tumor Growth, Cell Migration, and Response to PI3K Inhibitor. Mol Cancer Ther 2018 Sep;17(9):2049-2059.