Author's response to reviews

Title: Pentastatin-1, a collagen IV derived 20-mer peptide, suppresses tumor growth in a small cell lung cancer xenograft model

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Author's response to reviews: see over
We respectfully submit our revised manuscript “Pentastatin-1, a collagen IV derived 20-mer peptide suppresses tumor growth in a small cell lung cancer xenograft model” for consideration for publication in *BMC Cancer*. We would like to acknowledge and express our appreciation for the constructive comments given by reviewers, and feel our revised manuscript effectively addresses all concerns raised upon our first submission. We provide a detailed response to the reviewers as follows:

**Reviewer 1:**

1. The reviewer suggested a BrdU or thymidine incorporation assay to show pentastatin-1’s effects on the proliferation of NCI-H82 and 3T3 fibroblast cell lines. We provide a revised Figure 1 including a BrdU incorporation assay showing the effects of pentastatin-1 on NCI-H82 small cell lung cancer cells and mouse 3T3 fibroblast cells, in addition to the previous viability assay using WST-1 reagent.

2. The reviewer asked us to include additional immunostaining for proliferation and apoptosis in vivo. Figure 4 has also been revised to include caspase-3 immunohistochemical staining showing the effects of pentastatin-1 on apoptosis in vivo. We observed no quantitative difference in proliferation between the control and pentastatin-1 at 5 mg/kg by Ki67 proliferation staining.

**Reviewer 2:**

1. The reviewer raised the question of the toxicity of the peptide on the xenograft-bearing mice. To address this, we refer to a previous publication, accepted in *Neoplasia*, (Koskimaki, JE et. al, *Neoplasia*. in press) where we tested the toxicity of pentastatin-1 in breast xenograft-bearing severe combined immunodeficient mice (SCID) on liver, lung, and kidneys by H&E staining. We viewed no qualitative differences or systemic toxicity due to peptide administration in either control or xenograft-bearing mice.

2. The reviewer asked us to include a survival study in the concern that many anti-tumor agents have ultimately failed in clinical trials due to failure to prolong survival. We understand the importance of this issue, however, we are unable to complete such a study using the NCI-H82 cell line, as this rapidly growing cell line would not metastasize before growing to unethically large sizes grown on the flank of the animals. This suggestion has been noted for future work as we pursue development and optimization of the peptide in different xenograft models.
We believe our results are significant, and to be of interest to researchers studying peptide-based therapies as treatment for angiogenesis-dependent diseases.

We want to thank the reviewers for their constructive comments, as well as the editorial staff at *BMC Cancer* for indicating the potential of this manuscript as acceptable for publication.

Thank you for your time and consideration for publishing this manuscript.

Sincerely,

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