MTORC-1 Inhibition by Meclizine in Canine Osteosarcoma Cells

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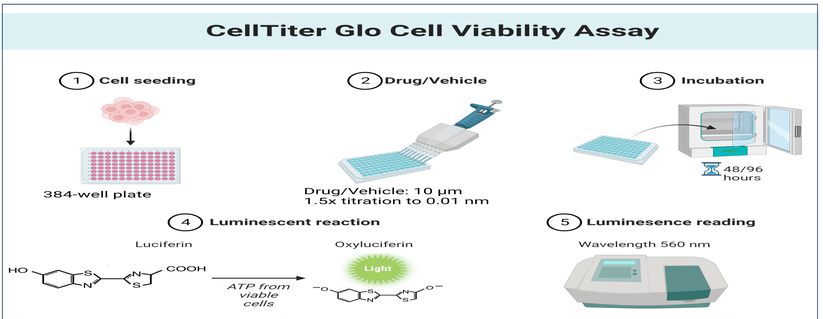
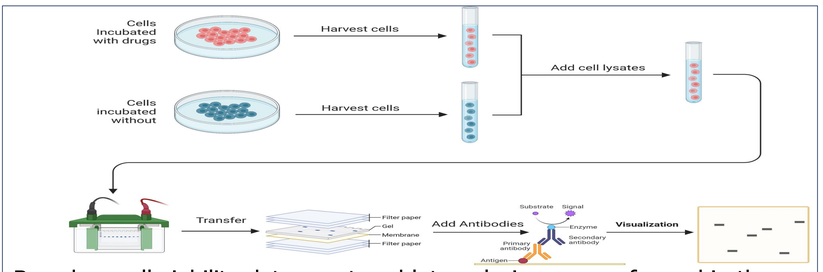
Conclusion and Future Directions

- Meclizine and other mTORC1 specific inhibitors **do not** kill canine osteosarcoma cells better than general mTOR inhibitor rapamycin.
- Rapamycin induced statistically significant killing in 2 of 3 of the osteosarcoma cell lines, but not to a substantial degree.
- Meclizine induced statistically significant killing in the same cell lines as rapamycin but to a lesser degree.
- Meclizine **does not** inhibit phosphorylated S6K or protein kinase B (AKT) in the most resistant osteosarcoma cell line whereas rapamycin inhibits phosphorylated S6K with some inhibition of AKT.
- These findings evaluated the potential of meclizine as an anti-neoplastic drug in a cancer derived from a different cell type and species with mixed results.
- Further research will investigate the mechanisms of why meclizine did not inhibit pS6K and find mTORC1 specific inhibitors in canine osteosarcoma.
- After finding an mTORC1 specific inhibitor, reperform cell viability assays in combination with standard of care chemotherapy drug (carboplatin, doxorubicin).
- Investigation of meclizine in canine glioblastoma cells can also be done for a more synonymous comparison between studies.

Methods



Osteosarcoma cells were incubated in presence of drugs for 48 or 96 hours. Subsequently cells were lysed and viability reagent was added. Cell viability was indirectly measured through the amount of ATP by luminescence.

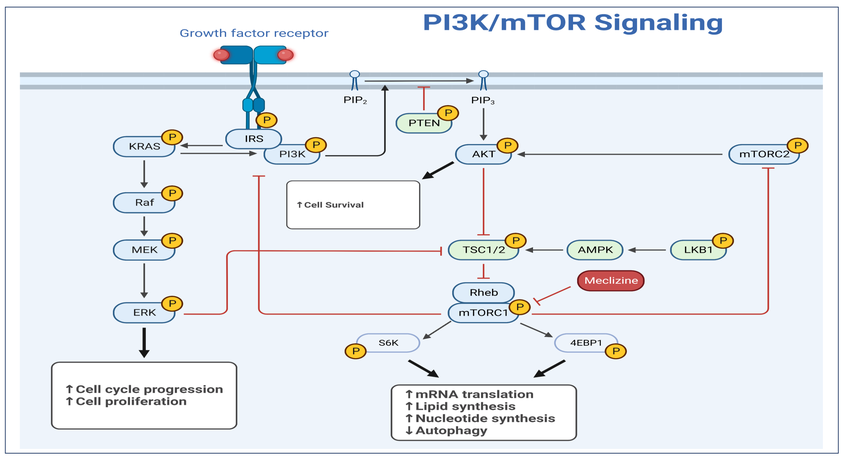


Based on cell viability data, western blot analysis were performed in the most resistant osteosarcoma cell line. Signal was analyzed to create a dose-response curve.

Introduction

Mammalian/mechanistic target of rapamycin (mTOR) is a downstream kinase of the phosphatidylinositol 3-kinase (PI3K) pathway. It has been shown that mutations in genes involving this signaling pathway is linked to several cancers in dogs and humans, although mTOR itself has not been shown to be mutated. mTOR consists of two distinct complexes known as mTORC1 and mTORC2, both have been shown to be dysregulated in cancer. Rapamycin inhibits mTORC1 initially and then proceeds to inhibit both complexes over time. When mTORC1 is activated, it promotes cell growth and proliferation mainly by phosphorylating downstream ribosomal protein 70S6Kinase (70S6K) and eukaryotic translation initiation factor 4E binding protein 1 (4EBP1). PI3K pathways has been shown to be altered in canine osteosarcoma. In a study 59 canine osteosarcomas (OSAs), 37% of the tumors had gene alterations involving the PI3K pathway. Past studies have also shown that inhibition of mTOR by rapamycin resulted in a dose-dependent decrease in colony growth and surviving fraction on canine OSA cells. Although rapamycin has been shown to be useful in inhibiting cancer cells, clinical trials have failed to show efficacy and its use in dogs has been limited by it's potential for toxicity. More recently, small molecules have been shown to specifically inhibit mTORC1 and not mTORC2, one of these being meclizine. It has also been shown that meclizine kills human glioblastoma cells better than rapamycin and in combination with standard of care temozolomide better than temozolomide alone.

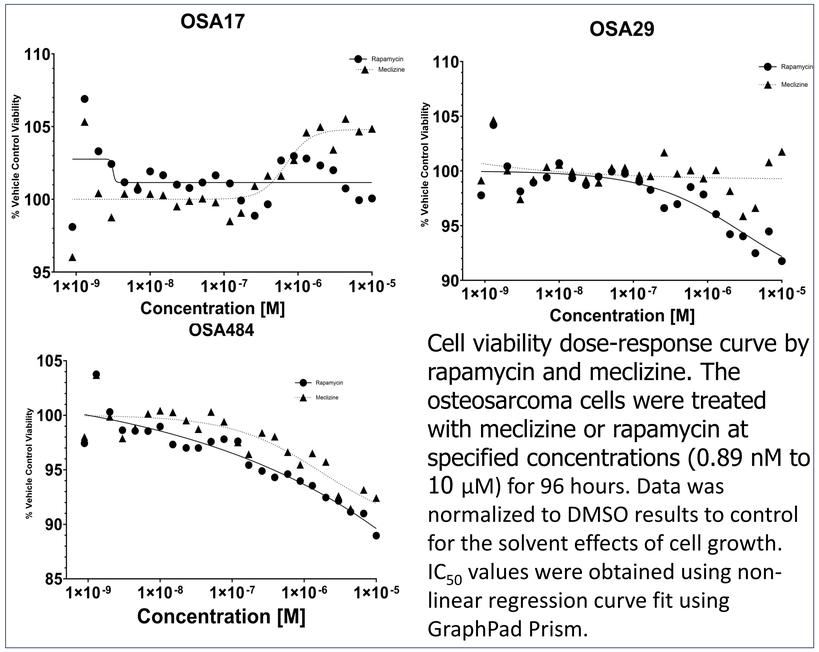
Pathway Diagram



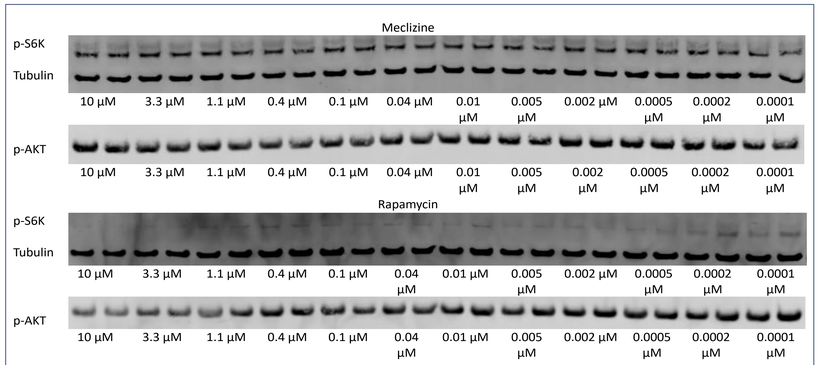
Hypothesis and Aims

- Hypothesis: Inhibition of mTOR results in a decrease in the phosphorylation of downstream p70S6K as well as a decrease in canine osteosarcoma cell survivability in cell viability assays. Meclizine that has been shown to shown to specifically inhibit mTORC1 better than rapamycin will decrease phosphorylated S6K without affecting AKT.
- Aim 1: Determine meclizine's effects on canine osteosarcoma cell survival at various concentrations vs rapamycin.
- Aim 2: Determine meclizine's effects on the mTOR and its effectors in canine osteosarcoma cells.

Results



Cell viability dose-response curve by rapamycin and meclizine. The osteosarcoma cells were treated with meclizine or rapamycin at specified concentrations (0.89 nM to 10 µM) for 96 hours. Data was normalized to DMSO results to control for the solvent effects of cell growth. IC_{50} values were obtained using non-linear regression curve fit using GraphPad Prism.



Most resistant cell line OSA17 were treated with each drug at specified concentrations for 1 hour. Proteins were then extracted and prepared for western blot analysis. Membranes were treated with validated antibodies against phosphorylated S6K and AKT. Tubulin was also included to ensure even protein loading.

Acknowledgements and References

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