MK-2206, a Novel Akt Inhibitor, Suppresses Medullary Thyroid Cancer Proliferation Independent of RET

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Introduction

Medullary thyroid cancer (MTC) is a neoplasm arising from calcitonin-producing parafollicular C cells. It is associated with a lower overall survival rate than well-differentiated epithelial thyroid cancers, and, although it represents only 5% of all thyroid cancers, it accounts for more than 13% of deaths from thyroid cancer.[1]

Many new therapies proposed for MTC target the RET protein, but the phosphoinositide-3-kinase (PI3K)/Akt pathway upregulation has also been linked to tumor growth in many cancer cell lines. Inhibition of the PI3K/Akt leads to growth suppression in multiple tumor types, including MTC.[2-5]

MK-2206 is a novel orally administered compound that allosterically inhibits Akt phosphorylation at serine 473 and threonine 308 residues. A phase 1 trial showed it can be administered safely to humans at clinically effective doses.[6]

Hypothesis

Inhibition of Akt activation in medullary thyroid cancer with MK-2206 may cause suppression of tumor cell growth and function.

References

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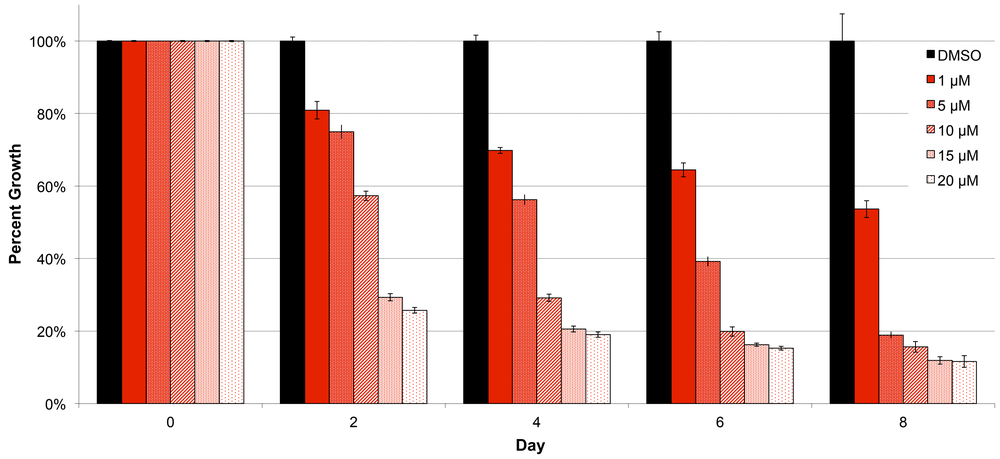
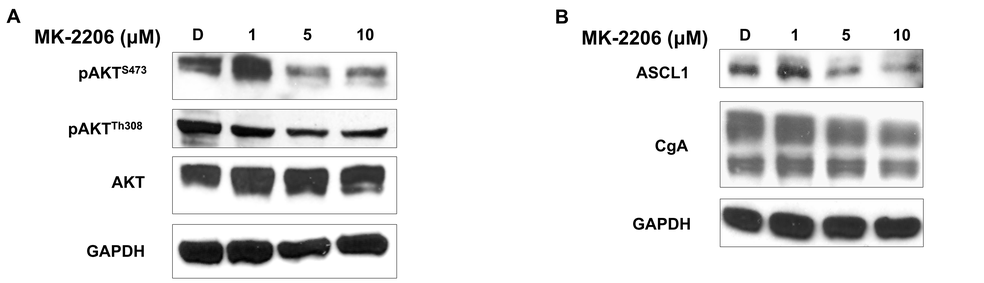
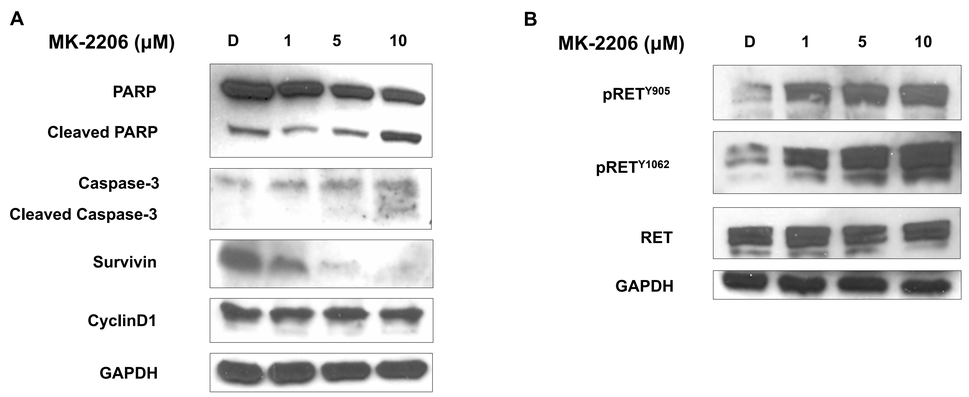
Results



Treatment with MK-2206 inhibits MTC-TT cell growth. MTT cellular viability assay demonstrates a dose- and time-dependent reduction in TT cell growth over 8 days. All represented doses are MK-2206 in cell media.



MK-2206 inhibits phosphorylation of Akt and reduces the levels of neuroendocrine tumor markers in MTC cells.
A) Increasing doses of MK-2206 reduce phosphorylation at the serine 473 residue of Akt, with more limited reduction at threonine 308. Levels of total Akt protein remain stable with treatment. B) MTC cells show a dose-dependent decrease in production of ASCL1 , with a marginal decrease in chromogranin A (CgA).



Growth suppression of MTC-TT cells by MK-2206 is mediated by apoptosis, and is independent of RET activity.
A) Treatment with MK-2206 resulted in increased cleaved PARP and cleaved caspase-3, with corresponding decrease in Survivin. Stable Cyclin D1 levels suggests no change in cell cycle signaling. B) Total RET protein levels are stable with MK-2206 treatment. Select phosphorylation residues actually increase with treatment, indicating RET activity is not blocked by MK-2206 treatment.

Methods

Treatment: MTC-TT cells, a stabilized cell line from human medullary thyroid cancer cells, were treated in culture medium with control (DMSO) or 1, 5, or 10 μM MK-2206 for 96 hours.

MTT Assay: The effects of MK-2206 on MTC-TT cell proliferation were measured by the methylthiazolyldiphenyl-tetrazolium bromide (MTT) rapid colorimetric assay. Viability of cells was measured after 2, 4, 6, and 8 days of treatment with varying doses of MK-2206 (1, 5, 10, 15, 20 μM).

Western Blotting: Protein lysates from MTC-TT cells treated with MK-2206 were analyzed. Mechanism of MK-2206 action was tested with $pAkt^{S473}$, $pAkt^{T308}$, and Akt, as well as downstream targets ASCL1 and CgA. Effect on the RET pathway was tested with $pRET^{Y905}$, $pRET^{Y1062}$, and RET. Mechanism of growth suppression was tested with apoptotic markers cleaved PARP and cleaved caspase-3, cell cycle marker Cyclin D1, and survival marker Survivin. Membranes were subsequently stained with GAPDH or β-actin to ensure equal loading of proteins.

Conclusions

- MK-2206 inhibits Akt in medullary thyroid cancer cells, leading to reduction in cell growth.
- Mechanisms are separate from the RET pathway. MK-2206 may be a treatment option for MTCs resistant to RET inhibitors.
- MK-2206 is easy to administer to humans, has been vetted in phase 1 trials, and thus could transition to phase 2 trials once its efficacy is proven for MTC treatment *in vivo.*

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