Co-Targeting the mTOR and MAPK Pathways is Effective in a Novel Mouse Model of Malignant Peripheral Nerve Sheath Tumors

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Abstract

Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are soft tissue sarcomas with low 5-year survival rates and no targeted therapies available. Data suggest that the mTOR and MAPK pathways may be involved in the formation and progression of MPNSTs, and both of these pathways can be inhibited with drugs that are currently in use for other tumor types. In vitro, RAD001 and PD-901, inhibitors of the mTOR and MAPK pathways, respectively, are effective at inhibiting proliferation of human MPNST cells, while having little effect on normal human Schwann cells. To better study their therapeutic potential, we tested these drugs in a mouse model of MPNSTs. This model closely resembles genetic changes (Pten loss, EGFR overexpression), and histological features of human MPNSTs. RAD001 or PD-901 treatment moderately reduced tumor burden and sizes, and extended lifespan in this model. However, when one pathway is inhibited, there is an increase in signaling through the other pathway, suggesting that these pathways feedback on one another, and that targeting both pathways in combination may be more effective. We found synergistic effects on reducing tumor burden and size, and a significant increase in lifespan when RAD001 and PD-901 are given in combination. The synergy seen is due to the combination therapy allowing for persistent and prolonged reduction in signaling through both pathways, without a subsequent increase in signaling through one pathway, as seen in single pathway treatments. These data suggest that co-targeting the mTOR and MAPK pathways could potentially be an effective treatment for patients with MPNSTs.

Background

1. Malignant Peripheral Nerve Sheath Tumors (MPNSTs)
   - Originate in the Schwann cell
   - Can occur spontaneously or in association with Neurofibromatosis Type 1
   - Low 5-year survival rate
   - Current Treatment: high dose, non-specific chemotherapy

2. Neurofibromatosis Type 1 (NF1)
   - Dominant genetic disorder
   - Occurs in 1 in 3000 live births
   - Causes benign tumor growth throughout entire peripheral nervous system

3. mTOR & MAPK pathways are activated in the development and progression of MPNSTs (Figure 1).
   - Loss of NF1 causes the mTOR and MAPK pathways to activate, which can lead to the transformation of benign neurofibromas to MPNSTs by inappropriate signaling.

4. Mouse model, Dhh-Cre; Ptenlox/lox, CNP-EGFR, closely resembles human disease (Figure 2)
   - Mouse model has loss of Pten and overexpression of EGFR in Schwann cells, which are common genetic changes in humans.
   - Major phenotypes include mobility problems and enlarged nerves; median lifespan: 28 days; average tumor burden: 13.7; tumor locations: dorsal root ganglia, intercostal nerves, trigeminal nerves, and lumbar plexus.
   - Good, testable model because of early onset and fast progression of disease.

Hypothesis

The mTOR and MAPK pathways are activated during the transformation of benign neurofibromas to MPNSTs, so by targeting these pathways with drugs we hope to improve disease by reducing tumor burden and grade, and prolonging lifespan. We will test this by treating mice with RAD001 and PD-901, which target the mTOR and MAPK pathways respectively. Additionally, due to feedback mechanisms between the pathways, a synergistic effect is expected using combination therapy in this model.

In Vitro Results

- **Figure 3**: MPNST cell lines are more sensitive to RAD001 and PD-901 compared to normal human Schwann cells.
  - This is important for targeting the MPNST cells, while leaving the normal Schwann cells unaffected. IC50s are in low micromolar range, making them physiologically relevant.

- **Table**: Combination therapy has synergistic effects in multiple MPNST cell lines.
  - Combination index shows how the drugs work together compared to either of the drugs used alone.

In Vivo Results

- **Figure 5**: Lifespan of mice treated with DMSO, a vehicle control, compared to RAD001 and PD-901 treatment.
  - (a) Treatment with RAD001 shows decreased signaling through the mTOR pathway, which is shown through decreased p-AKT and p-4E-BP.
  - (b) Treatment with PD-901 shows decreased signaling through the MAPK pathway, which is shown through decreased p-ERK.
  - (c) Co-targeting both the mTOR and MAPK pathways simultaneously results in a reduction of signaling through both pathways.

Pharmacodynamics

- **Figure 4**: Combination index shows that combination therapy has synergistic effects in multiple MPNST cell lines.
  - Combination index shows how the drugs work together compared to either of the drugs used alone.

Conclusions and Discussion

- Experiments done in vitro show that RAD001 and PD-901 are both effective drugs for targeting Schwann cells, and that combination treatment is synergistic.
- Treatment with either RAD001 or PD-901 in vivo as single agents has modest therapeutic effects with regard to slowing down tumor growth and progression as well as extending lifespan.
- Combination therapy (RAD001 + PD-901) in vivo is better at slowing down tumor growth and progression as well as extending lifespan.
- Pharmacodynamics show the combination therapy is better at reducing the resistance to drugs at late time points.

Future Directions

- Other mouse model (Dhh-Cre; Nf1lox/lox, Ptenlox/lox)
  - Made to resemble NF1-associated MPNST development

- Other targeted therapies
  - Wnt inhibitors
  - In combination with chemotherapy (how human patients would likely receive the treatment in a clinical trial).

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References

- Avgousti, Christina. "Cytokines of TGF-β Development and Progression of Neural Nerve Tumors in Neurofibromatosis Type 1." *Future Oncol* 1:2, no. 6 (March 2004): 695-691.

Pharmacodynamics

- At late time points, the mTOR pathway gets reactivated, shown by small amounts of p-AKT and p-4E-BP at early stages followed by large amounts at late stages.
- At late time points, the MAPK pathway gets reactivated, shown by small amounts of p-ERK at early stages followed by large amounts at late stages.
- Pharmacodynamics: Combination therapy reduces incidence of resistance by keeping signaling through these pathways low for a larger period of time, shown by continued low levels of protein at late time stages.

Figure 6: Average number of dorsal root ganglion neurofibromas from each treatment group: DMSO, RAD001, PD-901, and combination therapy.

- Treating mice with both drugs simultaneously has a bigger effect with decreasing the average amount of neurofibromas than when treating the mice with either drug alone.
- Error bars indicate standard deviation; *p < 0.05, **p < 0.0001, unpaired T-test.

Figure 7: A comparison of dorsal root ganglion and sciatic nerves of each of the treatments to DMSO, a vehicle control, at the end life stages. When these drugs are used in combination, there are fewer MPNSTs and they are much less enlarged than when treated with DMSO or one drug alone. The sciatic nerve is also much smaller in mice treated with both drugs.