Combined inhibition of IGF-1R pathway and HDAC blocks Uveal Melanoma cell survival and induces apoptosis.

Joshua Abebe[1], Fatima Khan[2] and Chandrani Chattopadhyay[2]

Undergraduate at the University of Notre Dame[1], MDACC University Outreach Program[1], Melanoma Medical Oncology, UT MD Anderson Cancer Center[2]

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Introduction

Uveal Melanoma (UM) is a rare subtype of melanoma that grows from the melanocytes of the uveal tract of the eye. Although it's more treatable if the primary tumor is isolated in the uvea, the poor prognosis of this disease is caused by its metastasis to the liver. The metastatic UM has only one FDA approved therapy and needs new therapeutic strategies urgently.

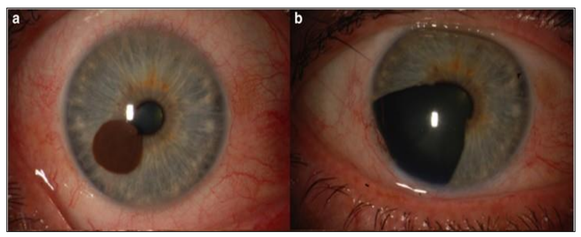

Fig 1. Depiction of tumor formation in uveal tract of the eye

Previously we've identified that inhibition of IGF-1R pathway using a targeting agent for insulin receptor substrate (IRS-1), inhibited UM cell survival and cell migration. IGF-1 is highly expressed in the liver (the primary site for UM metastasis), and the receptor IGF-1R is overexpressed in UM. Although NT157 (the IRS-1 inhibitor) showed inhibition of UM cell survival, we were interested in developing combination therapy strategies using this agent. Single agent therapies often do not work in melanoma in the long run. In a high throughput combination drug screen with NT157, we observed synergistic effect with NT157 and the HDAC inhibitor, Belinostat (Bel), in UM cell killing activity. To validate our findings from the drug screen, I performed combination treatments with NT157 and Belinostat on UM cell lines to determine their combined effect on UM cell survival and also the mechanism of such.

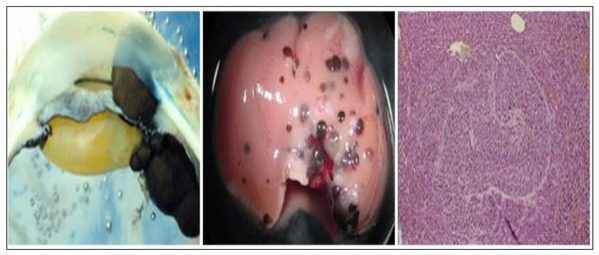

Fig 2. UM tumor metastasis seen in the liver and image of the affected liver cells

Methods

- We used multiple UM cell lines (MM28, MP38, 92.1, MP41, MP65, MEL202, and MEL20-06-039) with representative genotypes, monosomy 3 (metastatic UM genotype) and GNAQ/11 mutants of UM in these cell lines.
- The UM cell lines were cultured in RPMI 1640 media supplemented with 10% FBS, Penicillin-streptomycin, glutamine and HEPES.
- NT157, an inhibitor of IRS-1 and Belinostat, a histone deacetylase inhibitor (HDAC) were both purchased from SelleckChem.
- We assessed UM cell survival using MTT assays treated on cells with various concentrations of each agent. We also did colony formation assays for a confirmatory clonogenic assay depicting UM cell survival.
- Routine Western Blots (WB) were used for molecular analysis of the levels of protein markers specific to each pathway and apoptosis.

Results

High Throughput Drug Screen to predict novel combination therapy strategies for UM

Our initial high throughput combination drug screen with NT157 allowed us to predict a synergistic relationship between IGF-1R and HDAC inhibition in UM cell killing. A TargetMol Approved Drug library was used to test 2054 approved drugs for UM cell killing activity and eventually filtered down to 31 candidates for a combination screen. Belinostat was chosen for further investigation as it showed a good synergy score with NT157 combination (Figure 3).

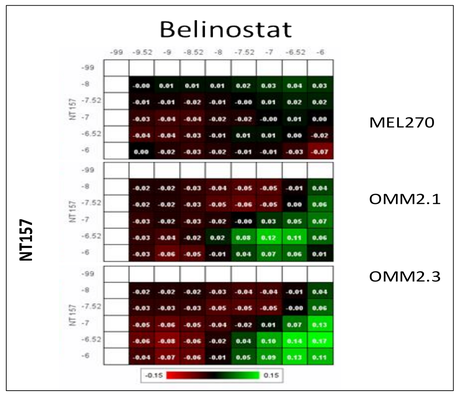

Fig. 3 Belinostat and NT157 combination shows high synergy scores from the combination drug screen.

UM cell survival is efficiently blocked with combined treatment using Belinostat and NT157.

Combination treatment with NT157 and Belinostat substantially improved the inhibition of UM cell survival over individual single agent treatments of UM cells as seen from MTT assays (Figure 4).

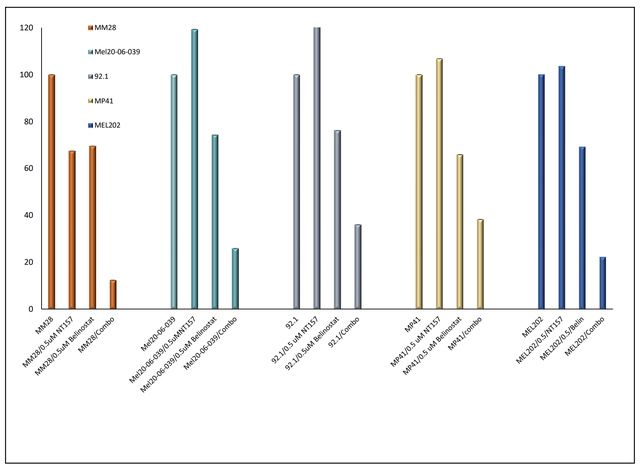

Fig. 4 Percent survival of monosomy 3 (MM28, MP41 and MEL20-06-039) and non-monosomy 3 (92.1, MEL202) UM cell lines under single agent and combination treatments

We further confirmed the effect of the combination treatment on UM cell survival using colony formation assays (Figure 5).

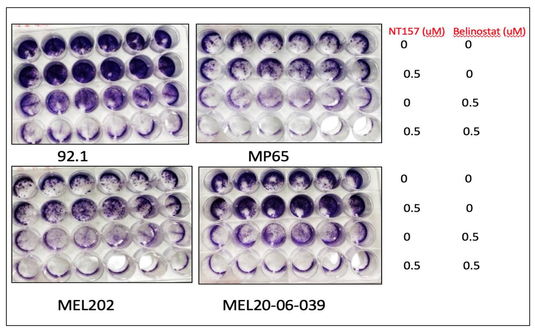

Fig. 5 UM cell lines with single agent and combination treatments in a colony formation assay.

Apoptosis was induced in UM cells treated with NT 157 and Belinostat in combination

The routine western blots indicated that individual protein markers of the respective target pathways were reduced on treatments with IGF-1R pathway inhibitor (IRS-1) and HDAC inhibitor (HDAC 3, Acetyl Histone H3) (Figure 6). Combination treatment also strongly induced apoptosis in the UM cells as evidenced from the increase in cleaved PARP levels (Figure 6).

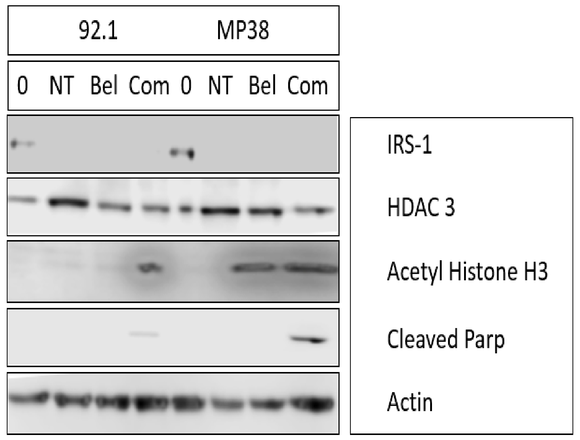

Fig. 6 Western blot analysis showing reduction of drug target levels /pathway marker proteins post treatment with single agents and in combination

Discussion

UM being an extremely rare form of melanoma and very different from cutaneous melanoma lacks established therapeutic measures. Our preliminary data shows IGF-1R inhibition to be successful in partial reduction of UM cell survival. Single agent therapies usually do not work in melanoma in the long run, which prompted our high throughput combination drug screen with IGF-1R pathway inhibiting agent NT157. From the drug screen, we found that inhibiting the HDAC pathway in combination with IGF-1R pathway can lead to synergy in UM cell killing.

To validate these findings I tested the above combination treatment on UM cell survival. I observed that UM cells respond better to the combination treatment as compared to the single agent treatments. I also tried to determine the mechanism of improved UM cell killing from the combined treatment with apoptosis assays. Western blots for cleaved PARP indicated apoptosis induction in UM cells as a result of the combination treatments.

The extent of response to the combination treatment is not the same in all cell lines and therefore in the future we plan to investigate the reason behind such differential responses. This would inevitably help improve the prognosis of this rare subtype of melanoma.

Conclusion

- IGF-1R pathway inhibition partially blocked UM cell survival
- Unbiased high throughput drug screen indicated synergy between IGF-1R and HDAC inhibition in UM cell killing activity.
- This was validated in *in vitro* UM cells based assays in the current study with nanomolar doses of each inhibitor
- Dual targeting of IGF-1R and the HDAC pathways improved UM cell killing over single agent treatments.
- Apoptosis was observed in low levels in the single agent-treated cells but even more for the combination treatments.

References

- Economou MA, All-Ericsson C, Bykov V, et al. Receptors for the liver synthesized growth factors IGF-1 and HGF/SF in uveal melanoma: intercorrelation and prognostic implications. Acta Ophthalmol. 2008;86 Thesis 4: 20-25.
- Reuveni H, Flashner-Abramson E, Steiner L, et al. Therapeutic destruction of insulin receptor substrates for cancer treatment. Cancer Res. 2013;73: 4383-4394.
- Histone Deacetylase Inhibitors Induce Growth Arrest and Differentiation in Uveal Melanoma. Solange Landreville, Olga A. Agapova, Katie A. Matatall, Zachary T. Kneass, Michael D. Onken, Ryan S. Lee, Anne M. Bowcock, and J. William Harbour. Clin Cancer Res; 18(2) January 15, 2012.