Activation of the Mitochondrial ClpP protease is Synthetically Lethal with HDAC1/2 Inhibition in Glioblastoma Model Systems

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Summary

Background: Novel therapeutic targets are critical to unravel for recalcitrant malignancies, such as the most common primary brain tumor in adults, glioblastoma (GBM). Heterogeneity remains a hallmark of primary glial brain tumors and therefore targeting several pathways simultaneously is an appropriate approach. By integration of a transcriptome, metabolite and U-13C-glucose tracing analyses, we showed that activation of the mitochondrial ClpP protease by mutant ClpP (Y118A) or through utilization of second-generation imipridone compounds (ONC206 and ONC212) in combination with genetic interference of HDAC1 and HDAC2 as well as with global (Panobinostat) or selective (Romidepsin) HDAC inhibitors caused synergistic reduction of viability in established, neuro-sphere and patient-derived xenograft (PDX) cultures of human GBM, which was mediated by interference with tricarboxylic acid cycle activity and GBM cell respiration. This effect was partially mediated by activation of cell death with apoptotic features along with activation of caspases regulated chiefly by Bcl-xL and Mcl-1. Knockdown of the ClpP protease or ectopic expression of a ClpP D190A mutant substantially rescued from the inhibition of oxidative energy metabolism as well as from the reduction of cellular viability by ClpP activators and the combination treatment, respectively, suggesting critical involvement of this protein. Finally, utilizing GBM PDX models, we demonstrated that the combination treatment of HDAC inhibitors and imipridones reduced tumor growth and prolonged host survival more potently than single treatments or vehicle in vivo. Collectively, these observations suggest that the efficacy of HDAC inhibitors might be significantly enhanced through ClpP activators in model systems of human GBM.

Results

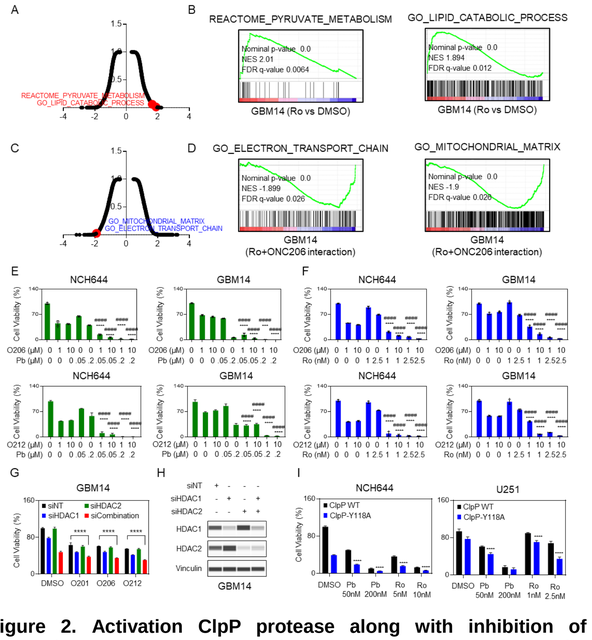

Figure 1. Activation ClpP protease along with inhibition of HDAC1/2 causes synthetic lethality in GBM model systems. (**A-D**) GBM14 cells were treated with 2.5 nM romidepsin for 24h and were submitted for microarray analysis followed by GSEA. Shown in (A, C) is a volcano plot (FDR-q vs. NES). Shown in (B, D) is the gene set enrichment analysis. NES: normalized enrichment score, FDR-q-value (n=2). (**E, F**) NCH644 and GBM14 were treated with ONC206/ ONC212 (1 μM and 10 μM) in the presence or absence of panobinostat (50 nM and 200 nM)/ romidepsin (1 nM and 2.5 nM) for 72h and cellular viability analysis was performed (n=5). (**G**) Cellular viability of GBM14 cells transfected with a siRNA against HDAC1, HDAC2, or combination of both in the presence or absence of ONC201, ONC206, or ONC212 (n=4). (**H**) Standard western blots of GBM14 cells transfected with a siRNA against HDAC1, HDAC2, or combination of both. Vinculin is used as a loading control. (**I**) ClpP-wild type or ClpP-Y118A U251 and NCH644 cells were treated with panobinostat/romidepsin for 72h and cellular viability analysis was performed (n=4). Statistical significance was assessed by ANOVA with Dunnett's multiple comparison test. *the statistic was compared between ONC206 and combination, # the statistic was compared between Pb/ Ro and combination. *p<0.05, ***/****p<0.001, #p<0.05,####p<0.001.

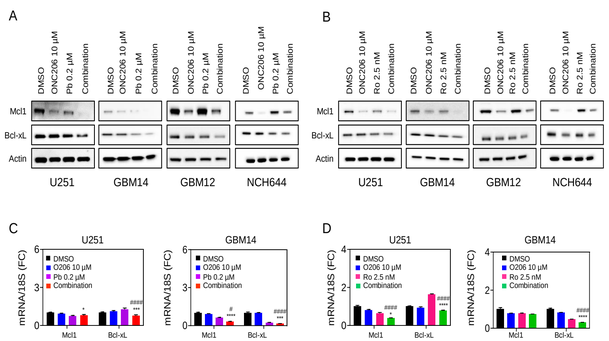

Figure 2. Activation ClpP protease along with inhibition of HDAC1/2 regulates the expression of anti-apoptotic Bcl-2 family members. (**A, B**) Standard western blots of Bcl2 family in U251, GBM14, GBM12, and NCH644 cells treated with 10 μM ONC206 and 0.2 μM panobinostat/ 2.5 nM romidepsin for 24h. (**C, D**) Real time PCR analysis of U251 and GBM14 cells treated with 10 μM ONC206 and 0.2 μM panobinostat/ 2.5 nM romidepsin for 24h. 18S is used as an internal control. Shown are mean and SD (n=4). Statistical significance was assessed by ANOVA with Dunnett's multiple comparison test. *the statistic was compared between ONC206 and combination, # the statistic was compared between Pb/ Ro and combination. *p<0.05, ***/****p<0.001, #p<0.05,####p<0.001.

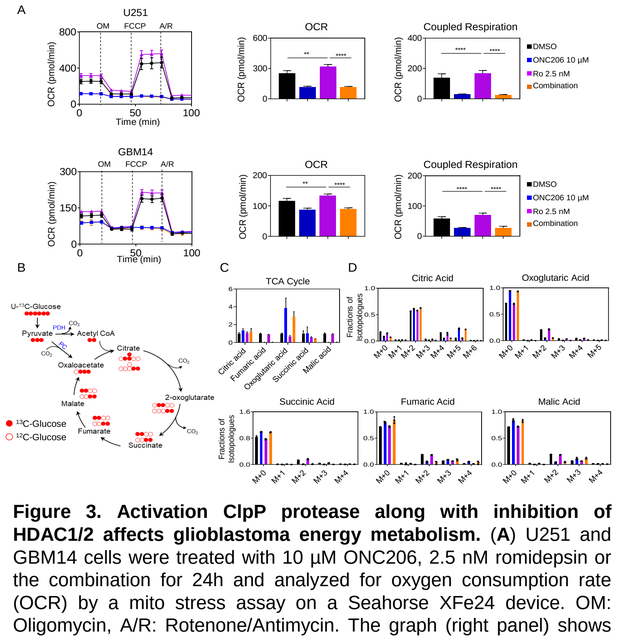

Figure 3. Activation ClpP protease along with inhibition of HDAC1/2 affects glioblastoma energy metabolism. (**A**) U251 and GBM14 cells were treated with 10 μM ONC206, 2.5 nM romidepsin or the combination for 24h and analyzed for oxygen consumption rate (OCR) by a mito stress assay on a Seahorse XFe24 device. OM: Oligomycin, A/R: Rotenone/Antimycin. The graph (right panel) shows the OCR and coupled respiration level (n=4-5). (**B**) The graphical presents one turn of the TCA cycle. Glucose is metabolized to pyruvic acid (m+3) (three carbons labeled). When glucose is oxidized in the TCA cycle, citric acid (m+2) is produced (two carbons are labeled). When glucose is used for anaplerosis, citric acid (m+3) is produced (three carbons are labeled). (**C, D**) GBM14 cells were incubated in DMEM (devoid of phenol red, pyruvate and glutamine) supplemented with 25 mM U-13C-glucose, 4 mM glutamine, and 1.5% dialyzed FBS in the presence of 10 μM ONC206, 2.5 nM romidepsin or the combination for 24h. Shown in (C) are the total metabolites in the TCA cycle (n=3). Cells were harvested for LC/MS analysis the TCA cycle. Shownin (D) are the fractions of isotopologues (n=3). Statistical significance was assessed by ANOVA with Dunnett's multiple comparison test. **p<0.01, ****p<0.001.

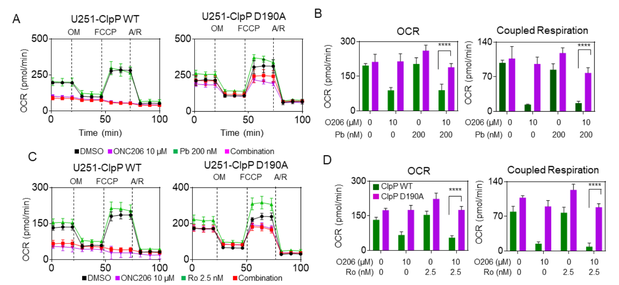

Figure 4. The mitochondrial protease, CLPP, is the metabolic effect mediator of the combination treatment of imipridones and HDAC inhibitors. (**A-D**) Stably transduced cells expressing ClpP-wild type or ClpP-D190A mutant treated with 10 μM ONC206, 0.2 μM panobinostat/ 2.5nM Romidepsin and the combination for 24h were analyzed for oxygen consumption rate (OCR) by mito stress assay on a Seahorse XFe24 device. OM: Oligomycin, F: FCCP, R/A: Rotenone/Antimycin. The graph in (B, D) show the OCR and coupled respiration level (n=4-5).

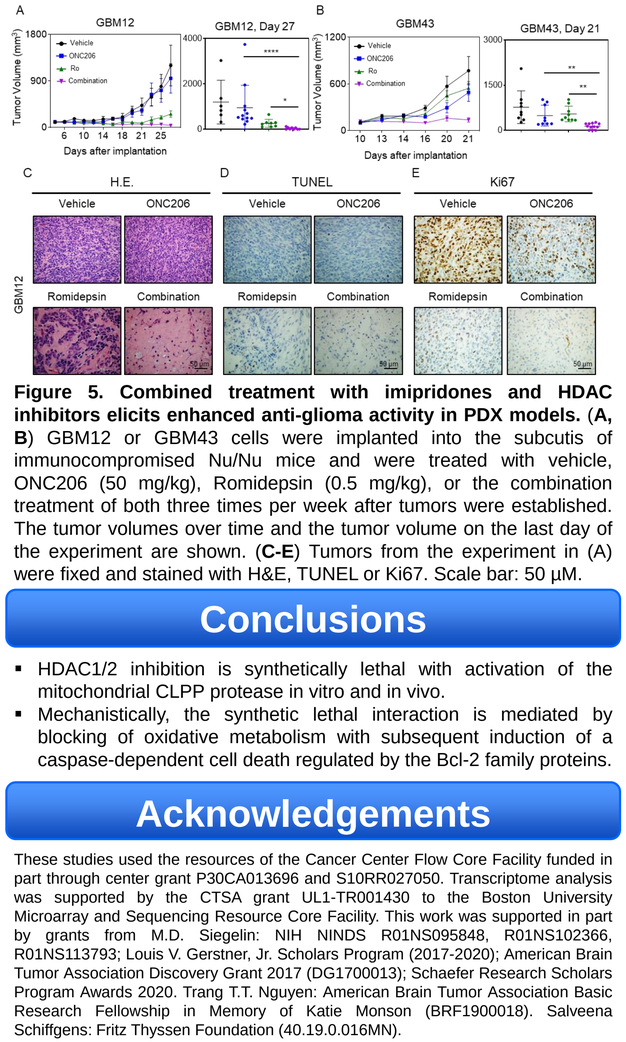

Figure 5. Combined treatment with imipridones and HDAC inhibitors elicits enhanced anti-glioma activity in PDX models. (**A, B**) GBM12 or GBM43 cells were implanted into the subcutis of immunocompromised Nu/Nu mice and were treated with vehicle, ONC206 (50 mg/kg), Romidepsin (0.5 mg/kg), or the combination treatment of both three times per week after tumors were established. The tumor volumes over time and the tumor volume on the last day of the experiment are shown. (**C-E**) Tumors from the experiment in (A) were fixed and stained with H&E, TUNEL or Ki67. Scale bar: 50 μM.

Conclusions

- HDAC1/2 inhibition is synthetically lethal with activation of the mitochondrial CLPP protease in vitro and in vivo.
- Mechanistically, the synthetic lethal interaction is mediated by blocking of oxidative metabolism with subsequent induction of a caspase-dependent cell death regulated by the Bcl-2 family proteins.

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Conflict of interest: Varun Prabhu and Josh Allen are employees and/or shareholders of Chimerix/Oncoceutics.