Targeting the Achilles' Heel of RAS Dysregulated Tumors

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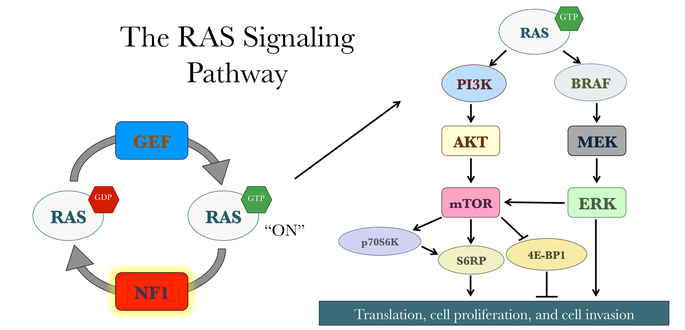
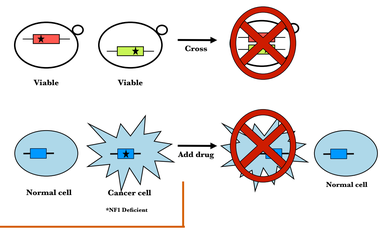
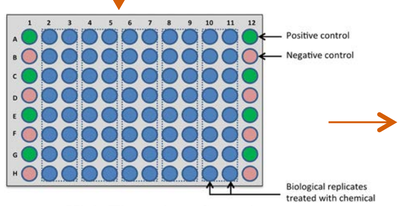
INTRODUCTION



Overview of Synthetic Lethality

Synthetic lethality was described in yeasts as a genetic interaction between two mutations in different genes. In a synthetic lethal pair, exclusive mutation of one gene does not impact cell viability; mutation of both, however, is fatal. Cancerous cells have mutations called "drivers" that are responsible for their tumorigenic phenotype. By identifying drugs that mimic mutations that are synthetic lethal with cancer drivers, it becomes possible to selectively target and kill cancer cells. Naturally, normal cells that do not contain the mutation are spared. This concept provides a framework for the development of novel cancer therapeutics.





High-Throughput Screening

Over 12,000 compounds were tested in wild-type and *ira2Δ* (homologue of NF1) yeast lines. Drugs that proved to be potent inhibitors of cell proliferation in mutant yeast were furthered screen in RAS dysregulated mammalian cell lines.. Compound 833 was one among these drugs.

In Vivo Testing using PDX Models

Xenograft tumor models will be established from patient-derived, NF1-deficient tumor tissue at low passage. Tool compounds selected from screens will be tested in vivo in mouse with RAS dysregulated tumor.

RESULTS

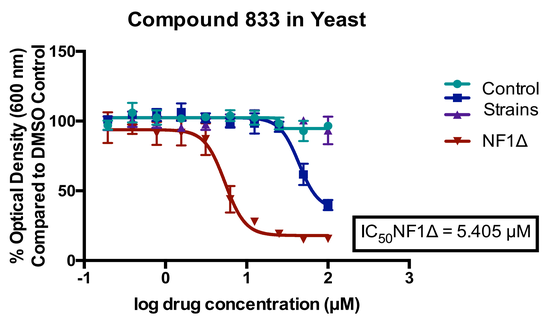


Figure 1. Selective sensitivity RAS dysregulated cells to compound 833. Yeast was seeded at 1000 cells per well on day zero. The cells were treated for 18 hours with compound 833 at various concentrations ranging from 1 x 10^{-7} M to 1 x 10^{-4} M. Cell growth was assessed by optical density in culture at 600 nm. Three hours prior to collection, cells were stain with AlamarBlue indicator dye. Plates were read at an excitation wavelength of 544nm and emission wavelength of 590nm.

RESULTS CONTINUED

Compound 833 induces DNA damage.

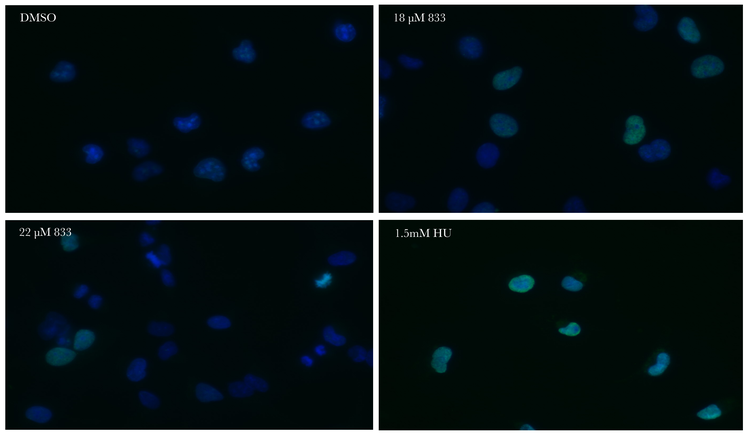


Figure 3. Detection of phosphorylated histone in isogenic WT IMECs. Wild type IMECs cells were plated at 50,000 cells per well and treated with DMSO, 18μm 833, 22μm 833, or 1.5mM HU for 24 hours. Cells were fixed prior to staining for γ-H2AX (green), a marker of DNA damage. DAPI was used to counterstain the nuclei (blue).

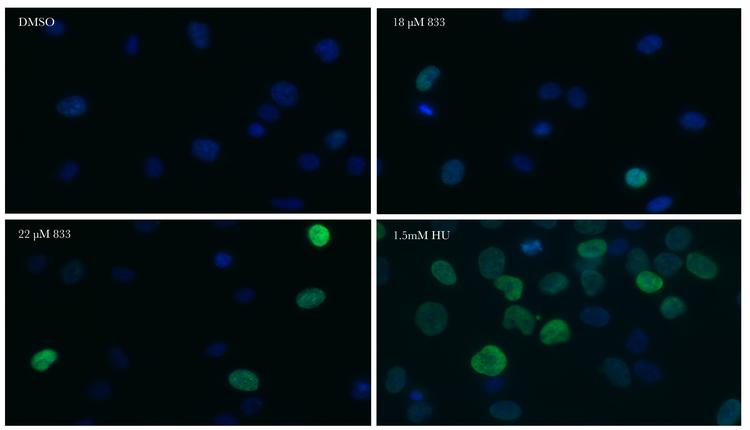


Figure 4. Detection of phosphorylated histone (γ-H2AX) in isogenic mutant IMECs. Mutant IMECs cells were plated on coverslips at 50,000 cells per well and treated with DMSO, 18μm 833, 22μm 833, or 1.5mM HU for 24 hours. Cells were fixed prior to staining for γ-H2AX (green). DAPI was used to counterstain the nuclei (blue).

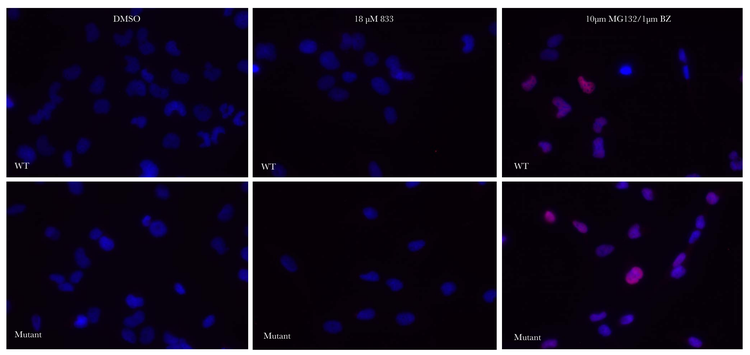


Figure 5. Absence of hypoxia-inducible transcription factor post 833 treatment. Wild type *(Top)* and mutant *(Bottom)* IMECs cells were plated on coverslips at 50,000 cells per well and treated with DMSO, 18μm 833, or 10μm MG132/1μm BZ for 24 hours. Cells were fixed prior to staining for HIF1-α (red), an indirect biomarker of reactive oxygen species. DAPI was used to counterstain the nuclei (blue).

PRELIMINARY CONCLUSIONS

- Compound 833 was revealed to be synthetic lethal with *ira2* yeast in high throughput screens.
- Compound 833 alters the nuclear cell phenotype of both wild type and mutant IMECs cells.
- Compound 833 affects cell viability by inducing DNA damage. This was suggested by the accumulation γ-H2AX biomarkers in the nucleus.
- Compound 833 does not cause accumulation of reactive oxygen species or induce hypoxic cellular conditions, as indicated by the lack of nuclear HIF1-α accumulation.

Hypothesis: Compound 833 inhibits proliferation in RAS dysregulated cells lines by generating chromosomal double-stranded breaks. In addition, tool compounds that selectively target *ira2* yeast will also target NF1 deficient mammalian tumor cells.

FUTURE DIRECTIONS

- Immunofluorescent staining shows a greater signal for chromosomal damage in mutant than in wild type IMECs cells. In order to confirm preferential inhibition of the IMECs mutant, it is necessary to perform additional dose response assays with compound 833 with a 72 hour treatment time. The endeavor is currently in progress.
- Double strand breaks are one of many forms of chromosomal damage that can lead to cell arrest and death. Additional experiments can be performed to determine if 833 induces other types of DNA damage.
- Compound 833 does not induce DNA damage by causing hypoxic cellular conditions or by causing an accumulation of reactive oxygen species. Whether the drug induces other forms of oxidative stress, however, is unknown and must still be tested for in post-treatment cells.
- The targeted pathway(s) of compound 833 includes but is not limited to DNA damage. NF1 deficient tumors are dependent on other internal processes that if disrupted, could also lead to decreased viability. It would be of great interest to test whether 833 operates through mechanisms of actions other than chromosomal damage.

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