Investigation of novel pharmacological vulnerabilities of 9p21-deleted bladder cancer cells

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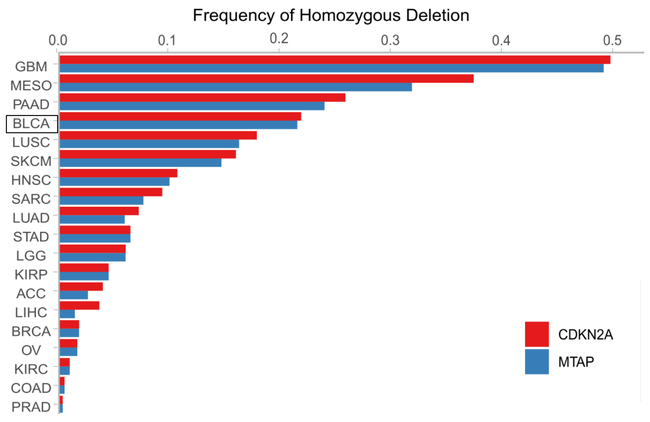
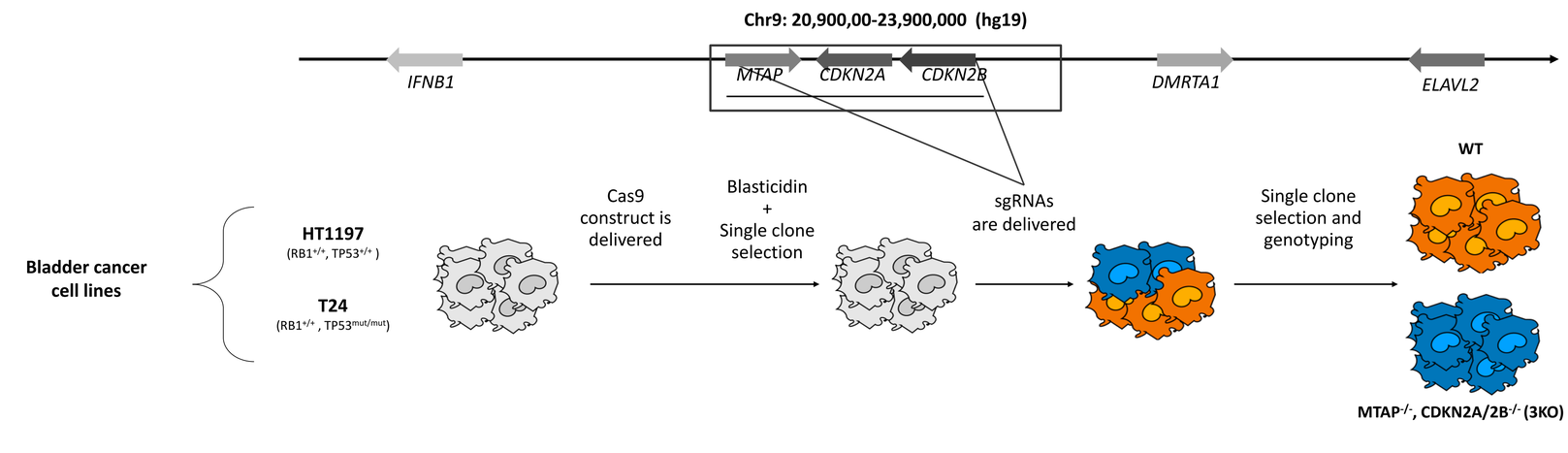
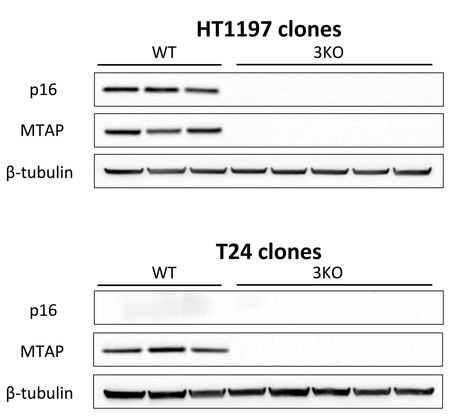
Background

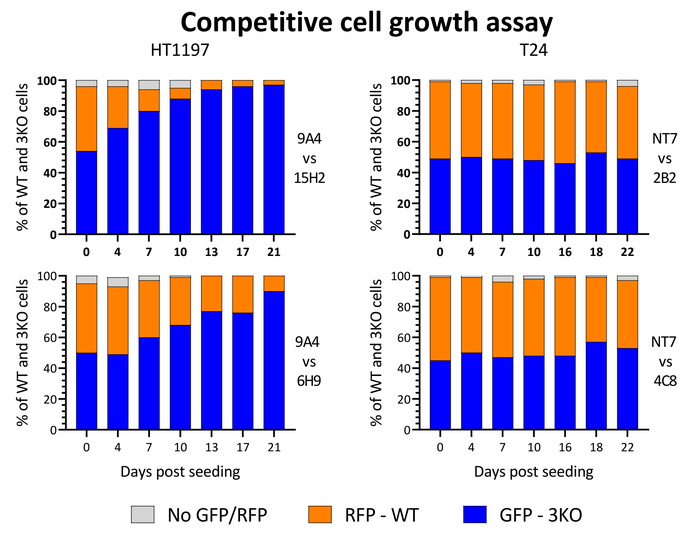
Deletion of the chromosome 9p21 locus is the most frequent copy number alteration in bladder cancer, identified in 23% of the TCGA muscle-invasive bladder cancer cohort[1]. It causes loss of the tumor suppressors *CDKN2A/2B* and of the metabolic gene *MTAP*, involved in the methionine and adenine salvage pathway. Large-scale shRNA screens have shown enhanced dependency of *MTAP*-deleted cells on PRMT5 and MAT2A, which led to the development of highly specific inhibitors (i.e. MRTX1719 for PRMT5 and AG270 for MAT2A)[2-6]. Here, we performed a multi-parametric drug screening to uncover new pharmacological vulnerabilities of 9p21-deleted bladder cancer cells, a disease with limited treatment options in the advanced stages[7].

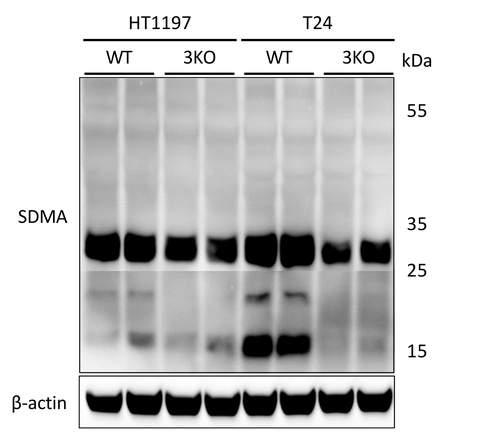


Generation and characterization of 9p21 locus isogenic pairs







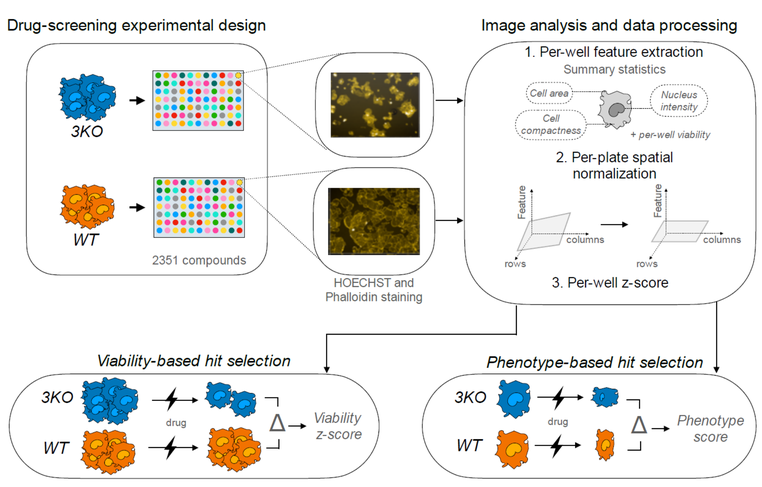


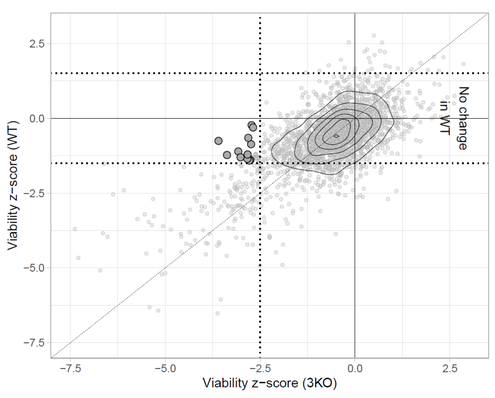
A. Several WT and 3KO clones derived from HT1197 and T24 cell lines have been isolated and characterized

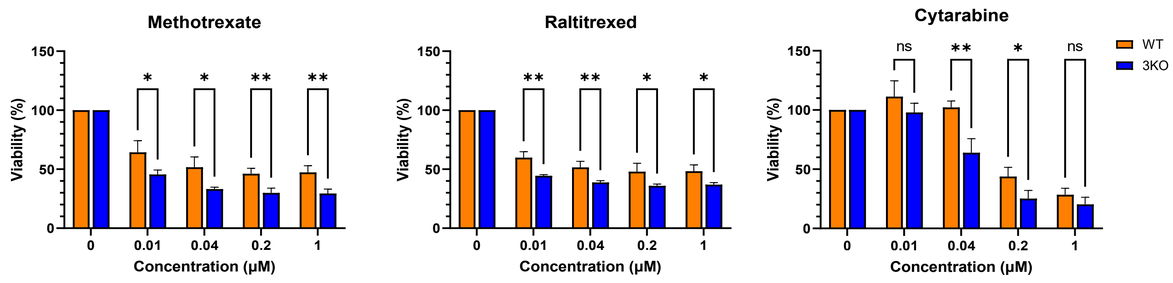
B. HT1197 3KO clones have higher proliferation rates compared to WT clones, while T24 clones proliferation is not altered by 9p21 loss

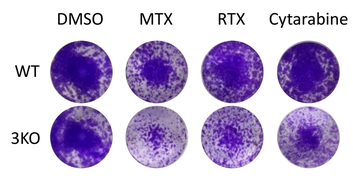
C. SDMA levels are lower in 3KO clones respect to WT clones; PRMT5 activity is partially impaired in HT1197 and T24 3KO clones

Drug screening workflow and validation



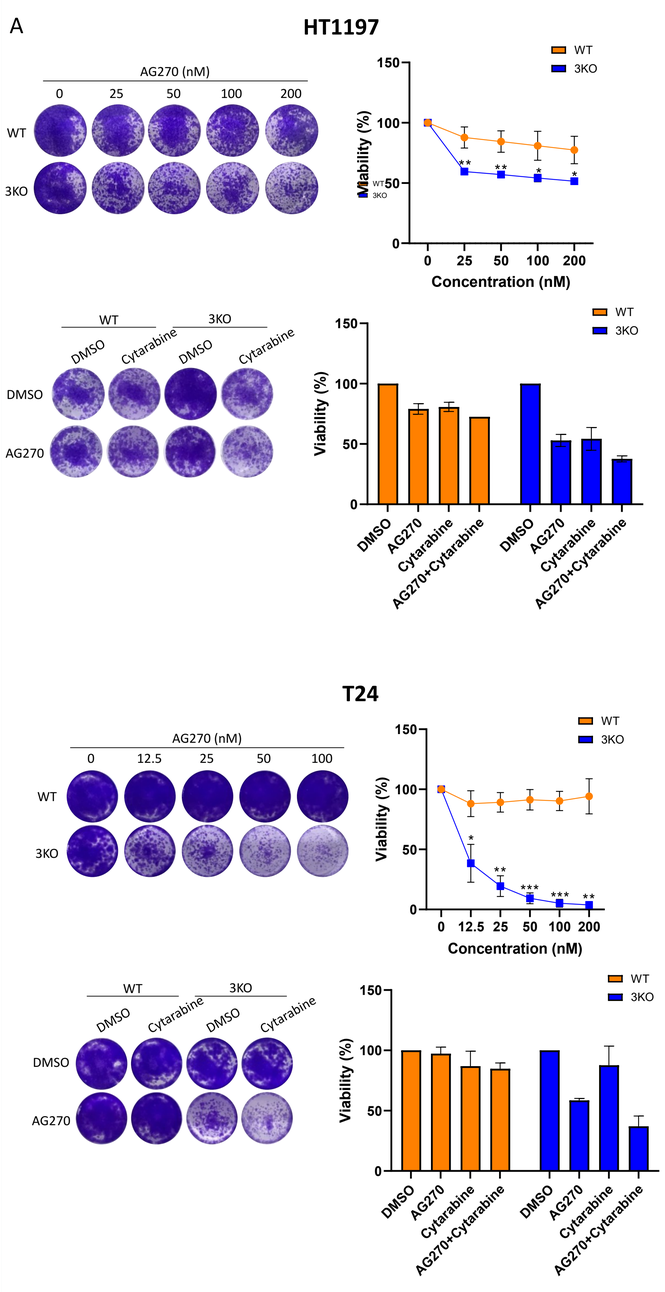


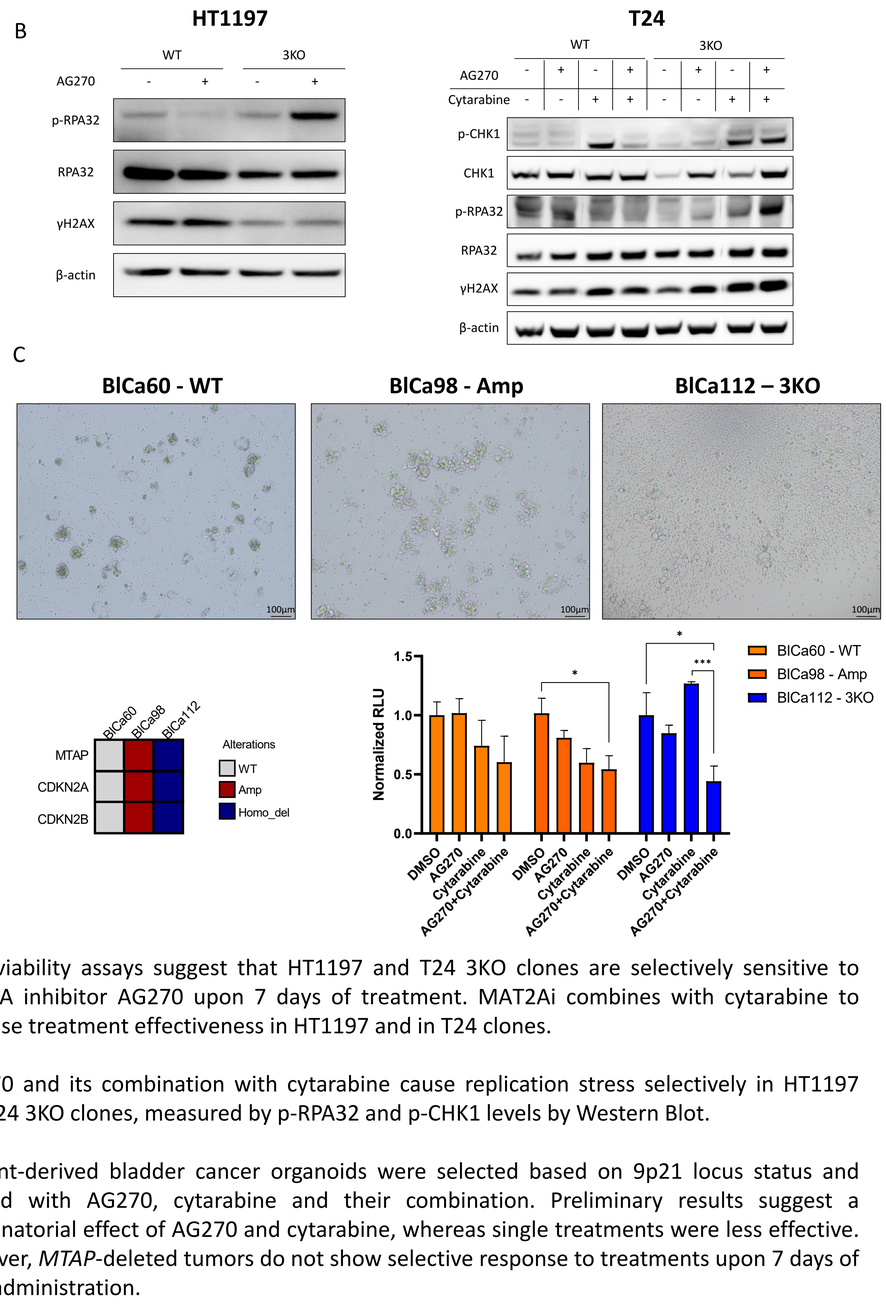




- The HT1197 isogenic pair was used to perform a multi-parametric drug screening testing 2351 compounds.
- 18 drugs that selectively affected 3KO clone cell number were found.
- Methotrexate (MTX), raltitrexed (RTX) and cytarabine were confirmed in dose-response viability assays performed with several HT1197 WT and 3KO clones.

Dependency of 9p21 KO cells on MAT2A





A. Cell viability assays suggest that HT1197 and T24 3KO clones are selectively sensitive to MAT2A inhibitor AG270 upon 7 days of treatment. MAT2Ai combines with cytarabine to increase treatment effectiveness in HT1197 and in T24 clones.

B. AG270 and its combination with cytarabine cause replication stress selectively in HT1197 and T24 3KO clones, measured by p-RPA32 and p-CHK1 levels by Western Blot.

C. Patient-derived bladder cancer organoids were selected based on 9p21 locus status and treated with AG270, cytarabine and their combination. Preliminary results suggest a combinatorial effect of AG270 and cytarabine, whereas single treatments were less effective. However, *MTAP*-deleted tumors do not show selective response to treatments upon 7 days of drug administration.

Conclusions and future plans

We successfully generated isogenic bladder cancer cell lines (9p21 locus WT and 3KO) that recapitulate literature findings in terms of proliferation rate, reduced SDMA levels and sensitivity to MAT2A inhibition. Our drug screening nominated two antifolates agents (MTX and RTX) and cytarabine as therapeutic vulnerabilities of *MTAP*-deleted cells. Our findings are in line with a recent study showing that the antifolate agent pemetrexed is selectively effective in *MTAP*-deficient bladder cancer patients and preclinical models[8]. AG270 effectively combines with cytarabine in isogenic bladder cancer cells. Preliminary results on patient-derived bladder cancer organoids suggest a combinatorial effect of AG270 and cytarabine but do not show clear genotype selectivity.

References

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