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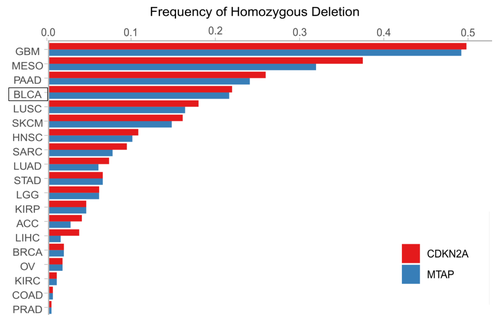
Exploration of therapeutic vulnerabilities exposed by 9p21 loss in bladder cancer cell lines

R. Bevilacqua[1], P. Gasperini[1], T. Cantore[1], T. Fedrizzi[1], V. Adami[2], M. Pancher[2], B.M. Faltas[3], F. Demichelis[1,4]

[1]Department of Cellular-Computational and Integrative Biology, Università degli Studi di Trento, Trento, Italy
[2] HTS Facility, Department of Cellular-Computational and Integrative Biology, Università degli Studi di Trento, Trento, Italy
[3]Departments of Medicine, Cell and Developmental Biology, Weill Cornell Medicine, New York, USA
[4]Englander Institute for Precision Medicine-New York-Presbyterian Hospital, Weill Cornell Medicine, New York, USA

1 Background

The deletion of chromosome 9p21 locus is the most frequent homozygous deletion in bladder cancer[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].



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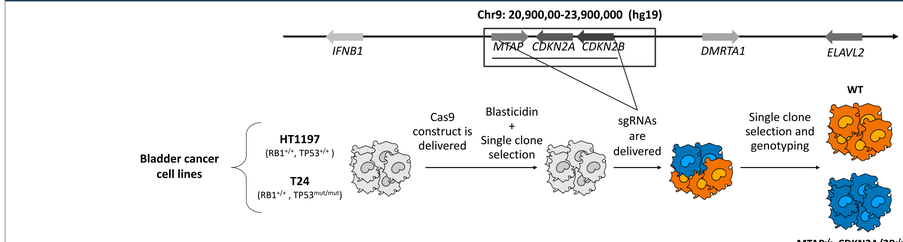
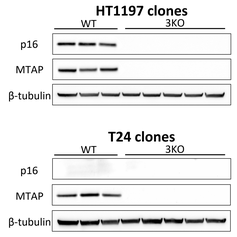
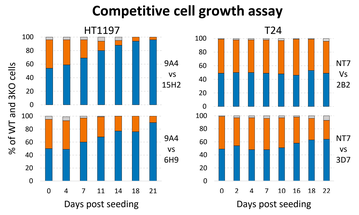
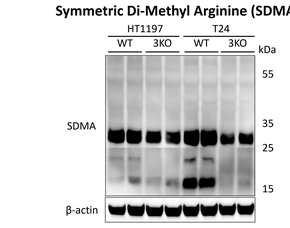
2 Generation and characterization of 9p21 locus isogenic pairs





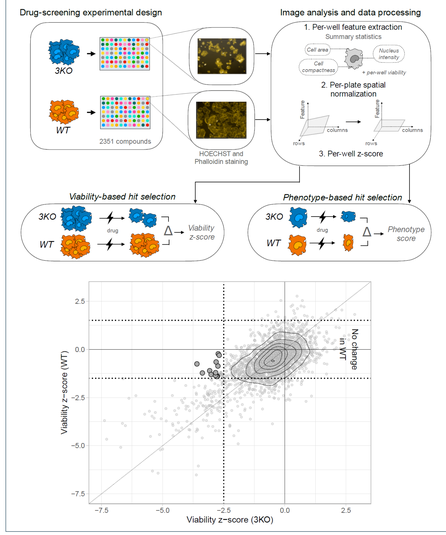


HT1197 3KO clones have higher proliferation rates compared to WT clones, while T24 clones proliferation is not altered by 9p21 loss

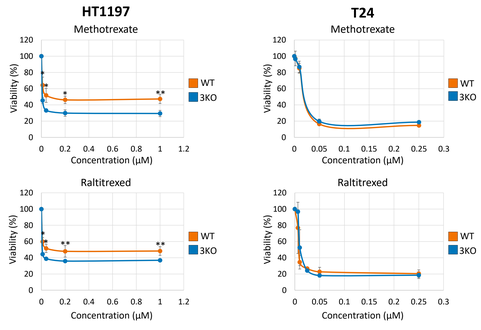


SDMA levels are lower in 3KO clones respect to WT clones; PRMT5 activity is partially impaired in HT1197 and T24 3KO clones

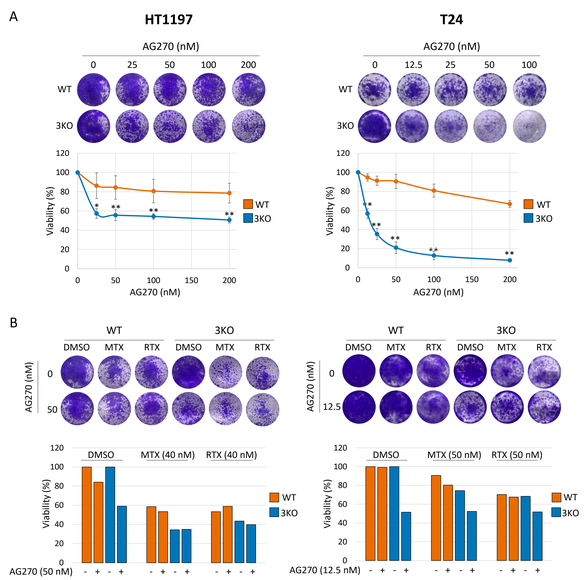
3 Drug screening workflow and validation



- 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) and raltitrexed (RTX) were confirmed in dose-response viability assays performed with several HT1197 WT and 3KO clones.
- However, T24 3KO clones do not show enhanced sensitivity to MTX and RTX treatment compared to WT cells.

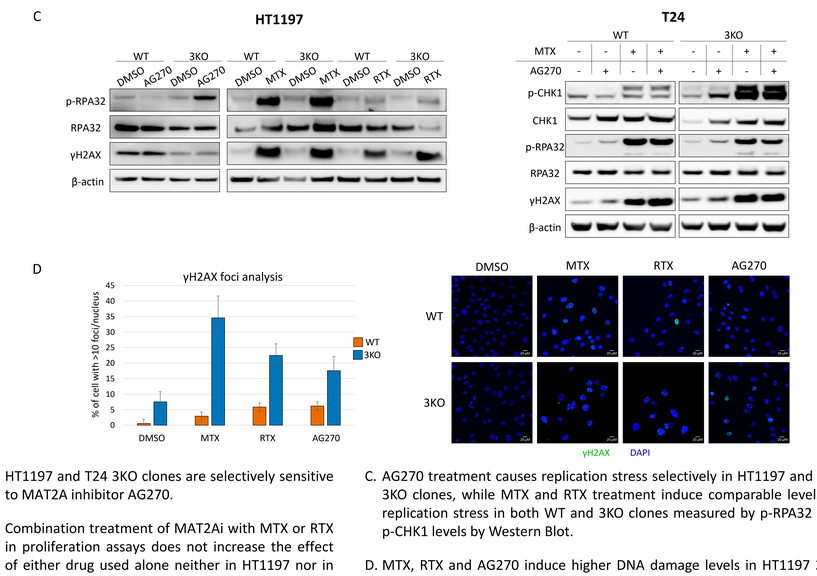


4 Dependency of 9p21 KO cells on MAT2A



A. HT1197 and T24 3KO clones are selectively sensitive to MAT2A inhibitor AG270.

B. Combination treatment of MAT2Ai with MTX or RTX in proliferation assays does not increase the effect of either drug used alone neither in HT1197 nor in T24 clones.



C. AG270 treatment causes replication stress selectively in HT1197 and T24 3KO clones, while MTX and RTX treatment induce comparable levels of replication stress in both WT and 3KO clones measured by p-RPA32 and p-CHK1 levels by Western Blot.

D. MTX, RTX and AG270 induce higher DNA damage levels in HT1197 3KO cells compared to WT when considering γH2AX foci formation by IF.

5 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 the antifolates agents MTX and RTX 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]. We are currently testing new combinations of drugs in order to increase treatment effectiveness in *MTAP*-deleted cells and patient-derived organoids.

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Contact: Riccardo Bevilacqua, Ph.D. candidate, Laboratory of Computational and Functional Oncology, University of Trento (riccardo.bevilacqua@unitn.it)