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Panel LS4

Intercellular trading in nucleotide metabolism: an emerging target

InterMet

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1. Abstract

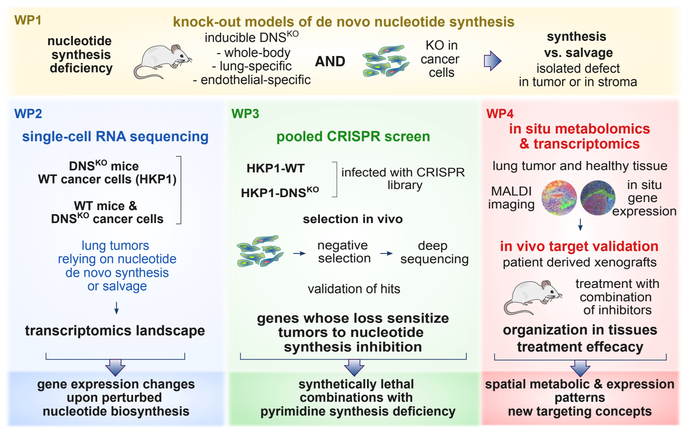
Anticancer therapy is >70 years old, and nucleotides are the oldest target in cancer treatment. Despite its long history, this treatment suffers high rates of resistance and toxicity. What are the reasons? A cell can gain nucleotides via de novo synthesis (DNS) or from salvage pathways. DNS inhibition can be bypassed by nucleotides produced by surrounding cells or distant organs, causing resistance. Cancer cells were traditionally studied in isolation, with (bulk) techniques precluding identification of cell type-specific targets, causing toxicity.

To date, the cellular sources of nucleotides in healthy and tumor tissues are poorly characterized. Can the complexity of metabolic crosstalk in tissues be captured by the traditional means?

I hypothesize that cancer and stromal cells differ in how they utilize nucleic acid building blocks from external and internal sources. A single cell resolution is needed to disentangle their interactions, and inhibition of both DNS and cancer-specific salvage is required for a successful blockade. I aim to define the nucleotide sources in heathy tissues and tumors, characterize their adaptations to DNS blockade to uncover the network of metabolic interactions in tissues and find effective and specific combinations of targets.

To reach this goal, I will use a unique combination of single cell multi-omics and tailored mouse models, an expertise and tools that I took the lead to set up. I will selectively disable DNS in the stroma (host mouse) and in cancer cells (syngeneic lung tumors) to generate tumors dependent on internally or externally produced nucleotides. In an integrative approach using spatial and single cell transcriptomics & metabolomics in situ, and functional genetic screen, I will search for targetable metabolic vulnerabilities of DNS-disabled cancer cells.

This innovative research opens the path to understanding the organization of tissue metabolic homeostasis for new personalized metabolism-based anticancer medicine.



2. Nucleotide metabolism: the oldest, yet elusive target in cancer

A

Flexibility of nucleotide metabolism

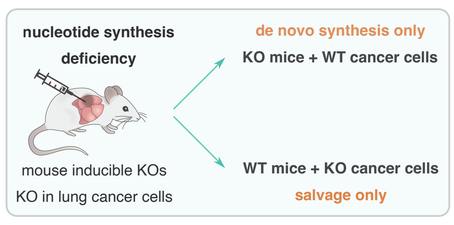
Nucleotides are essential for all proliferating cells. Cell can gain nucleotides in two ways: through **de novo synthesis** and **salvage** pathways.
Inhibition of de novo synthesis can be bypassed by nucleotides produced by neighboring cells or distant organs

Treatment by antimetabolites targeted at nucleotide metabolism is linked with **high rates of resistance and toxicity**.

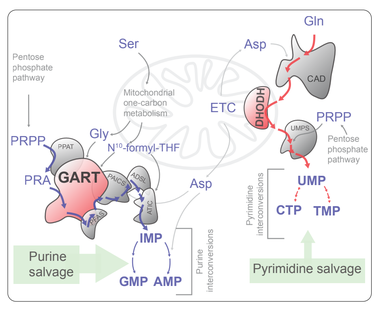
Research questions:

What are the molecular reasons for resistance?
What are cellular sources of nucleotides in tissues?
How is organized intercellular metabolic crosstalk in tumors?

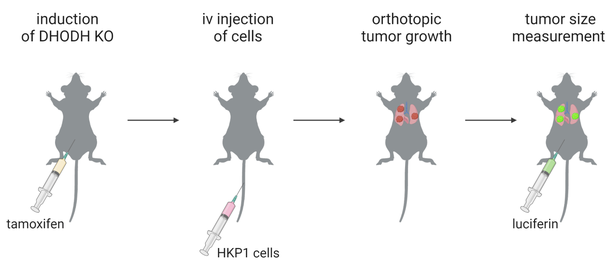
Approach: de novo synthesis vs. salvage



Nucleotide metabolism

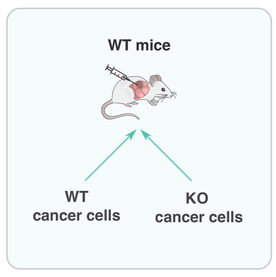


Tumor model



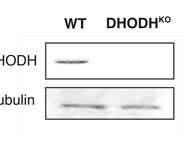
3. De novo synthesis is a major source of pyrimidines in tumors

A



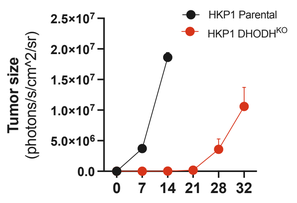
B

HKP1 WT and DHODH KO cells



C

Tumor growth of HKP1 WT and DHODH KO cells
WT mice

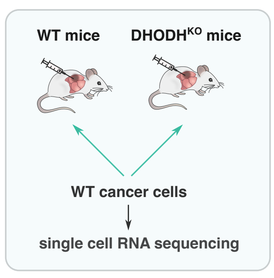


Tumor growth of cancer cells is in part supported by pyrimidine salvage from the environment: A, Experimental design. **B,** DHODH KO in HKP1, syngeneic lung adenocarcinoma cells. **C,** Tumor growth of HKP1 WT and DHODH deficient cells measured by luminiscence.

While de novo synthesis is a major source of pyrimidines in tumors, DHODH deficient cancer cells must be able to gain nucleotides externally

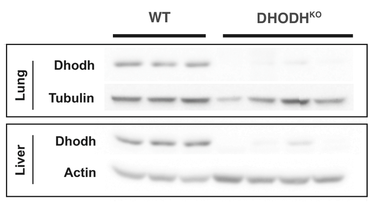
4. Pyrimidine production in tumor environment is tumor suppresive

A



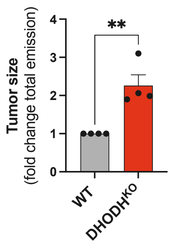
B

Mouse model of DHODH deficiency
whole body KO



C

Tumor growth of HKP1 WT cells
WT and DHODH KO mice



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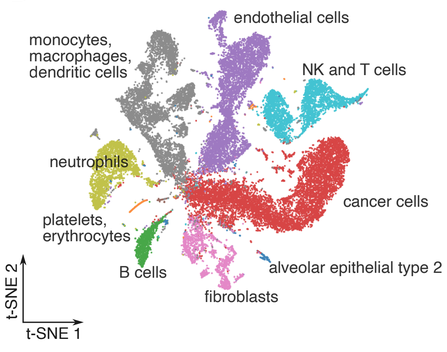
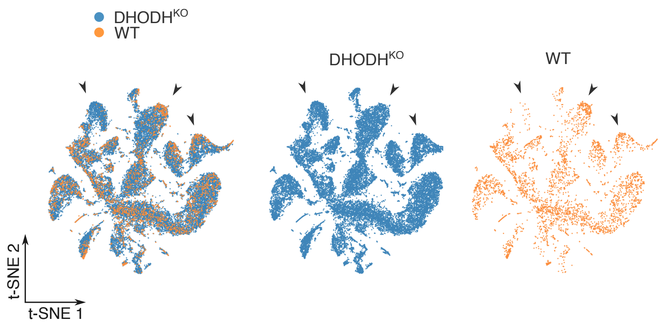


E

Which cell type is responsible for the accelerated tumor growth?



DHODH deficient tumor environmnent is more permissible for tumor growth: A, Experimental design. **B,** Whole-body mice with inducible DHODH^{KO} 32 days post induction. **C,** Tumor growth of HKP1 cells in DHODH WT and KO mice measured by luminiscence. **D,** t-SNE plot color-coded for cell clusters in lung tumors from WT and DHODH KO mice (D) and for the sample type (E). Arrowheads point to differences in transcriptomes in KO and WT mice.

5. Conclusions and open questions

DHODH KO cancer cells form tumors, albeit with a delay compared to WT cancer cells.

Mice with whole-body DHODH KO show accelerated orthotopic lung tumor growth.

DHODH deficiency in the tumor environment has the largest effect on endothelial and immune cells.

Despite reduced angiogenesis, EC-specific DHODH KO accelerated the growth of lung tumors.

Single cell RNA-seq and flow cytomentry showed major changes in the immune cell compartment of tumors from EC-specific DHODH KO mice.

Effectiveness of DHODH-targeted therapy on cancer cells may be compromised by its impact on ECs that promotes tumorigenesis, possibly contributing to resistance.

How can DHODH KO cells grow without de novo synthesis of pyrimidines?

How does DHODH deficiency in ECs impact the repertoire of immune cells in tumors?

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