Dietary Iron Levels Have No Effect on Pancreatic Cancer Growth in Mouse Model

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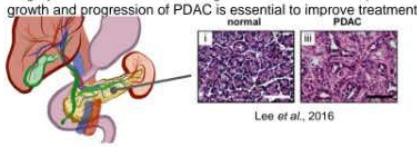
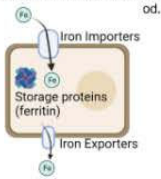
Background

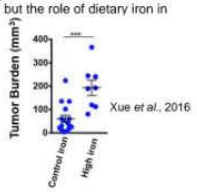
Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic cancer. PDAC has a poor prognosis since it is often detected late and spreads rapidly. Currently, there are few effective treatment options besides surgery, so a better understanding of the mechanisms responsible for the growth and progression of PDAC is essential to improve treatment.



Lee *et al.*, 2016

Iron plays a central role in many biochemical processes in the body. Iron can be regulated in cells through importers, exporters, and storage proteins (like ferritin). Previous work from our group shows that a high iron diet increases the number of tumors in a mouse model of colorectal cancer, but the role of dietary iron in od.





Xue *et al.*, 2016

Methods

3 weeks | 3 weeks

Start iron diets
- **Low = >5ppm**
- **Med = 35ppm**
- **High = 1000ppm**

Orthotopic tumors

Collection
- **Inductively coupled plasma mass spec (ICPMS; Fig 1)**
- **Immunohistochemistry (Fig 2-4)**

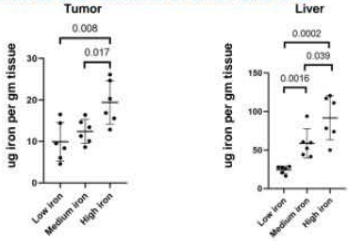
Mice (3 male, 3 female per diet) were pretreated with iron diets for 3 weeks before establishing orthotopic tumors in the pancreas with a mouse PDAC cell line. After the tumors grew for 3 weeks, we collected tissue for analysis.

Hypothesis

High dietary iron will cause PDAC tumors to grow larger and low dietary iron will cause PDAC tumors to shrink

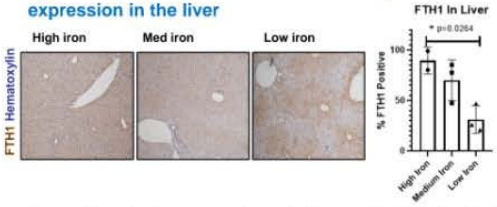
Results

Figure 1. Iron variable diets change total iron concentrations within the tumor and liver



Total iron levels (free and protein-bound) were measured in tissues using ICPMS. The diets are effective at altering tissue iron levels.

Figure 2. Low iron diets decrease ferritin (FTH1) expression in the liver

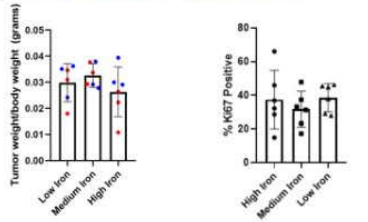


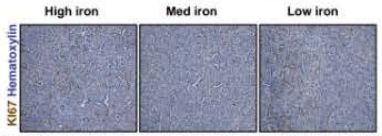
Immunohistochemistry was performed with an antibody against ferritin heavy chain (FTH1). The liver is an important organ in iron homeostasis. These results suggest that iron diets are also affecting the way cells are using and storing iron.

Figure 3. FTH1 levels in pancreas tumors are relatively similar across iron diets

Immunohistochemistry for ferritin showed similar levels across the tumors. This is in contrast to ferritin levels in the liver. This means the tumor maintained iron levels.

Figure 4. High and low iron diets do not significantly change tumor size or tumor proliferation





Tumor weight to body weight ratios were compared across diets and across sex (male=blue, female=red). Immunohistochemistry for proliferation marker KI67 was also unchanged across tumors. This suggests that tumors grew the same, regardless of dietary iron levels.

Conclusions

- ICPMS can be used to measure iron levels in organs
- Our use of iron diets was effective at changing total iron levels in the liver and tumor
- PDAC tumors did not change in growth or proliferation in response to iron levels, which means our hypothesis was not supported
- Our results suggest that PDAC has a way to overcome fluctuations in dietary iron

Future Directions

- We will look at expression of iron importers that could be used to maintain iron pools
- Repeat the diet experiment in a spontaneous mouse model of PDAC (rather than the orthotopic model)
- Treatment with an iron chelator in addition to a low iron diet to reduce iron levels even more
- Determine the role of other metals in pancreatic cancer

References

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- Thanks to Heather Giza for her organ drawing and for review of the poster

Resistance to bacteriophage in *E. coli* impacts antibiotic sensitivity

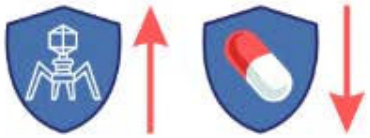
Maya Zheng, Kevin Crowthers PhD.

Background

Overuse of antibiotics results in **antibiotic resistance,** which causes more severe infections and creates a need for alternatives.

Phage therapy is an alternative that uses a special virus called a bacteriophage to kill bacteria (phages ONLY kill bacteria).

Some phages can reduce antibiotic resistance through evolutionary trade-offs ⇒ bacteria develop phage resistance and lose antibiotic resistance.



Researchable Question

How can phage T4 impact antibiotic resistance in E. coli by selecting for phage resistance mechanisms that cause an evolutionary trade-off with antibiotic resistance?

Hypothesis

If *E. coli* evolve resistance to phage T4, then the bacteria will display decreased resistance to colistin and vancomycin and increased resistance to ampicillin due to modifications to the phage T4 receptors outer membrane protein C (OmpC) and lipopolysaccharide (LPS).

Exposing *E. coli* to bacteriophage T4 results in **increased phage resistance** and **decreased antibiotic resistance.**

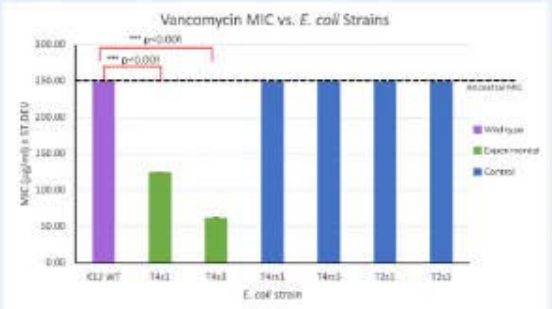


Figure 1. MIC comparison of vancomycin between the wild-type E. coli K12 and all mutant E. coli strains. Brackets represent significant differences in MIC between the wild-type and mutant strains.

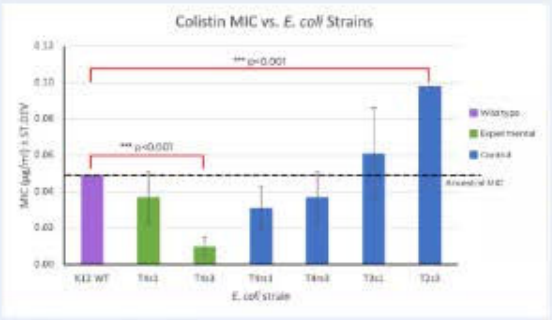


Figure 2. MIC comparison of colistin between the wild-type E. coli K12 and all mutant E. coli strains.

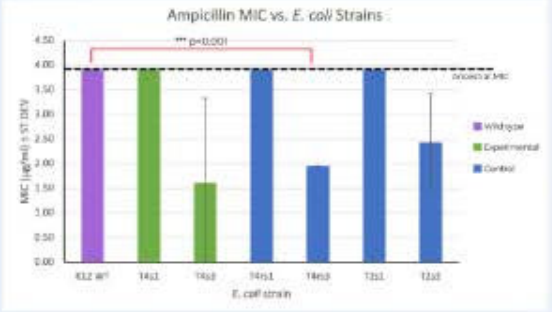
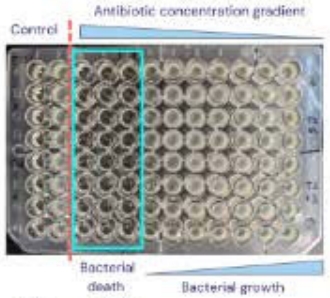


Figure 3. MIC comparison of ampicillin between the wild-type E. coli K12 and all mutant E. coli strains.

Methods

1) Determine the minimum inhibitory concentration (MIC) of vancomycin, colistin, and ampicillin for wild-type *E. coli* (Picture 1).
2) Expose *E. coli* to phage T4 overnight to develop resistance.
3) Confirm phage resistance with cross streak test (Picture 2).
4) Repeat MIC test with phage-resistant strains and compare (Picture 1).



Picture 1. MIC assay set up

Picture 2. Cross streak set up

Conclusions

- Phage T4-resistant *E. coli* strains had **significantly lower** MICs of **vancomycin and colistin** ⇒ decreased resistance.
- The MIC of ampicillin did not change significantly ⇒ unchanged resistance.
- Hypothesis partially supported ⇒ ampicillin MIC did not change significantly.

Future Work

- Use genetic sequencing to examine mutations in mutant *E. coli* strains.
- Repeat experiment with antibiotic-resistant *E. coli* ⇒ This experiment used wild-type due to safety restrictions.

SARS-CoV-2 viral rebound after Paxlovid treatment

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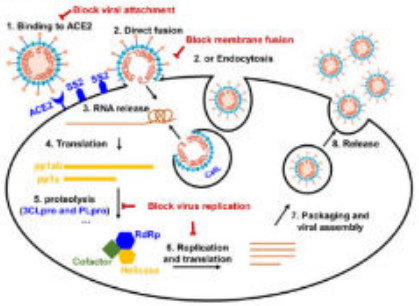
Background

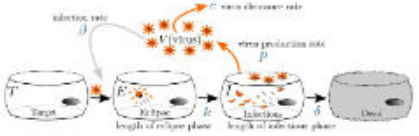
- COVID-19 is an infectious disease caused by the SARS-CoV-2 virus, which has resulted in over six million deaths.
- Paxlovid is a antiviral treatment for COVID-19 that is effective in preventing hospitalization.
- Paxlovid consists of two medications, nirmatrelvir and ritonavir, taken twice daily for 5 days.
- Currently, however anecdotal reports of rebound infection have been found with the use of Paxlovid.
- In this study we aim to use mathematical modeling to investigate the infection conditions that result in rebound of COVID-19 after antiviral treatment.

SARS-CoV-2 replication

SARS-CoV-2 is a single-stranded RNA virus. The spike protein on the viral capsid binds to the ACE2 receptor on the cell surface in order for the virus to gain entry to the cell. Once in the cell, the virus uses cell replication machinery to make copies of itself that are eventually released to infect other cells.



Mathematical model



In this model the target cells, T, are infected by the virus V, at a rate of β. Next these newly infected cells enter an eclipse phase, E. In phase E they are internally producing viral replicates but not releasing them. After a time $1/k$, the cell becomes productively infectious, I, and releases p infectious virions per unit time. Then the infectious virus decays at rate c.

$$\begin{aligned} \dot{T} &= -\beta TV \\ \dot{E} &= \beta TV - kE \\ \dot{I} &= kE - \delta I \\ \dot{V} &= pI - cV. \end{aligned}$$

Paxlovid

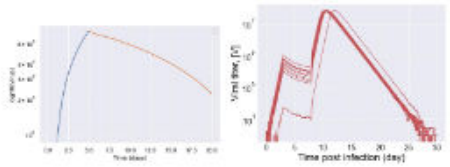
- Paxlovid consits of two separate medications, nirmatrelvir and ritonavir.
- Nirmatrelvir is a protease inhibitor. When the virus uses the cells machinery to replicate itself, it has to transfer its genome into a two large polypeptide proteins that then have to be broken down. The enzyme that breaks it down is SARS-CoV-2 is Mpro. Nirmatrelvir blocks Mpro, so it is unable bind to the polypeptide protein.
- This results in the virus being unable to create functioning protein and therefore being unable to replicate.
- Ritonavir is also a protease inhibitor and inhibits nirmatrelvir breakdown in the liver. This allows nirmatrelvir to have higher drug concentrations and stay in the body for a longer period of time.
- Since Paxlovid is a protease inhibitor it acts on part of the internal replication of the virus. So for our model we used the effect of Paxlovid as reducing the production rate of the virus.

$$p \rightarrow (1-\varepsilon)p,$$

where ε is the efficacy of the drug. An efficacy of 0 means the drug has no effect and 1 means the drug is 100% effective.

Stochastic simulations

- Stochastic modeling is a tool that brings randomness into the calculation.
- At each time step, a randomly chosen number of events occur, chosen based on the probability of those events happening.
- Therefore it accounts for random variance from patient to patient. This provides a more accurate depiction of the viral infection.



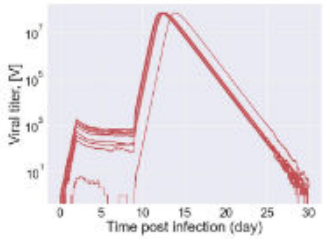
Simulating SARS-CoV-2

We used parameter values representing SARS-Cov-2 infection, taken from Hernandez-Vargas, 2020:

Parameter	Value
β	4.71×10^{-5} (copies/mL·d)$^{-1}$
p	3.07 copies/d·mL
k	5.0 /d
δ	1.07 /d
c	2.4 /d
V_0	0.31 copies/mL
T_0	4×10^8 cells

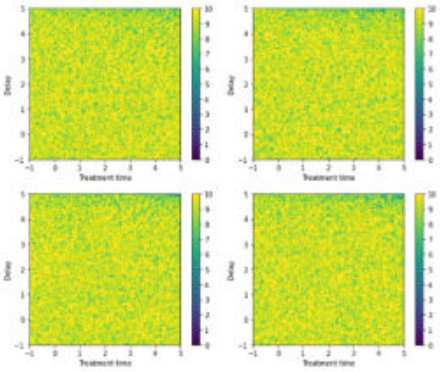
Methods

- We changed treatment time by setting up a range of 3 to 10 days in .07 increments in order to run simulations for different time durations.
- We changed treatment delay by setting a range of .5 to 7 days in .065 increments in order to simulate different delay times before treatment begins.
- For each combination of treatment time and duration, we simulated 10 patients then counted how many patients experienced rebound.
- We counted an infection as having rebound if the maximum viral load after treatment was 10% bigger than the last count during treatment.



Results for Paxlovid

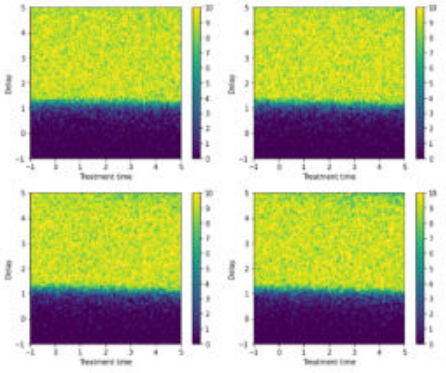
We simulate 10 patients at each treatment duration and treatment delay. The plots below show how many of the 10 had rebound at different drug efficacies.



- With Paxlovid viral rebound is virtually guaranteed no matter how effective the dose.
- This is because Paxlovid does not prevent infection of the cells, only blocks release of virus.
- Once the antiviral is removed, the infected cells that still remain start to produce virus again.

Results for a fusion inhibitor

We examined a drug that prevents infection to see if it was more effective at preventing rebound.



- Antivirals that prevent infection can prevent rebound if they are given early enough.
- The drug dose and the duration of treatment have little effect on the chance of viral rebound.
- If the drug is given too late, most cells have already been infected and the antiviral will not prevent rebound.

Conclusions

- Antivirals that disrupt processes after the cell has been infected are almost guaranteed to have viral rebound.
- Antivirals that prevent cell infection can prevent rebound, but only if the antiviral is given before too many cells are infected.
- The duration of treatment has little effect on whether there is rebound.

Future Directions

- Incorporate realistic time-varying drug treatment.
- Examine the relationship between infectious cell lifespan and treatment duration needed to prevent rebound.
- Model other antiviral mechanisms of action.

References

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