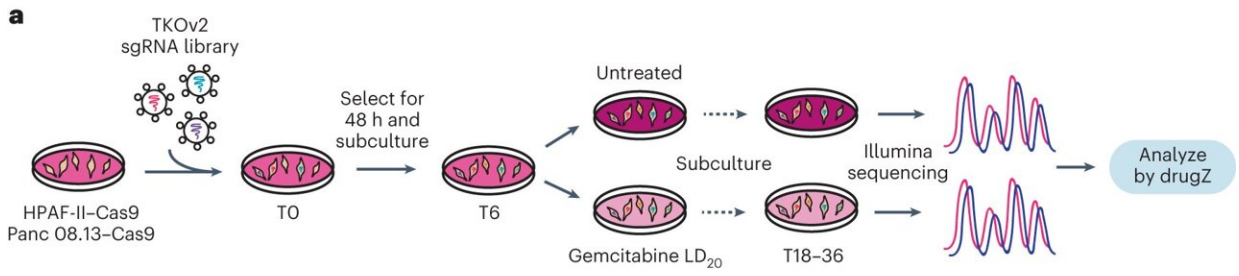
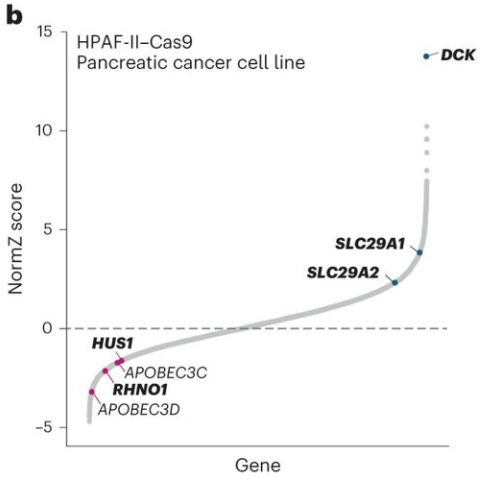
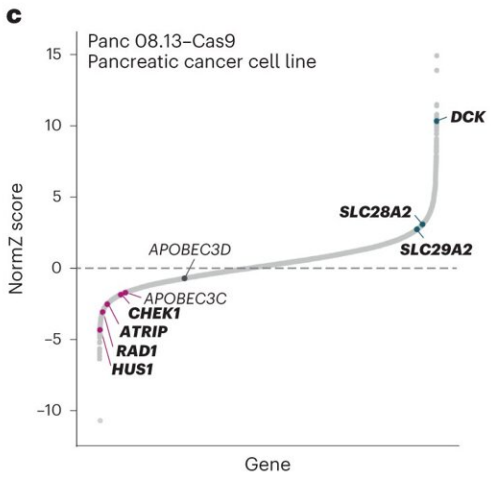
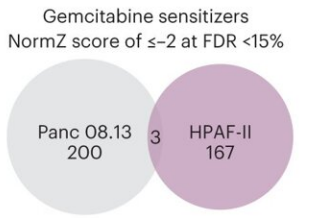
Researchers pinpoint issue that could be hampering common chemotherapy drug

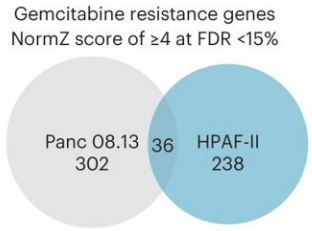
March 18 2024, by Anika Hazra

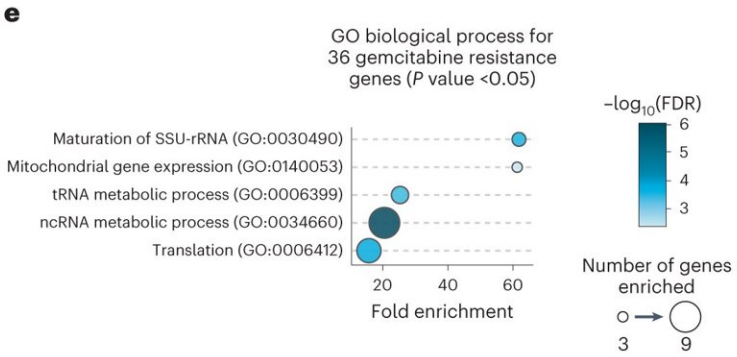

a
TKOv2
sgRNA library
HPAF-II–Cas9
Panc 08.13–Cas9
T0
Select for
48 h and
subculture
T6
Untreated
Subculture
Gemcitabine LD20
T18–36
Illumina
sequencing
Analyze
by drugZ

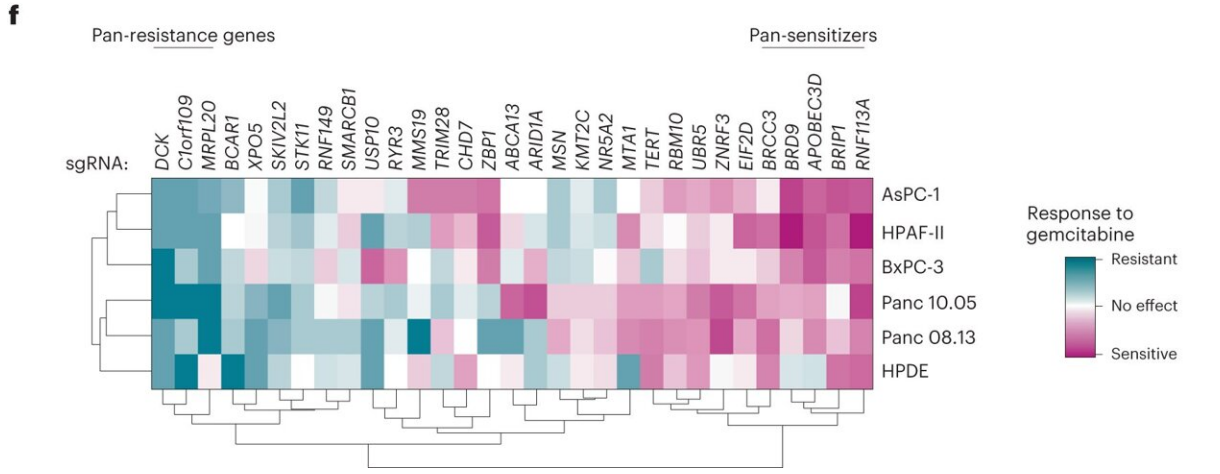

b
HPAF-II–Cas9
Pancreatic cancer cell line
NormZ score
Gene
DCK
SLC29A1
SLC29A2
HUS1
APOBEC3C
RHNO1
APOBEC3D


c
Panc 08.13–Cas9
Pancreatic cancer cell line
NormZ score
Gene
DCK
SLC28A2
SLC29A2
APOBEC3D
APOBEC3C
CHEK1
ATRIP
RAD1
HUS1


d
Gemcitabine sensitizers
NormZ score of ≤–2 at FDR <15%
Panc 08.13
200
3
HPAF-II
167


Gemcitabine resistance genes
NormZ score of ≥4 at FDR <15%
Panc 08.13
302
36
HPAF-II
238


e
GO biological process for
36 gemcitabine resistance
genes (P value <0.05)
Maturation of SSU-rRNA (GO:0030490)
Mitochondrial gene expression (GO:0140053)
tRNA metabolic process (GO:0006399)
ncRNA metabolic process (GO:0034660)
Translation (GO:0006412)
20
40
60
Fold enrichment
–log10(FDR)
6
5
4
3
Number of genes
enriched
3
9


f
Pan-resistance genes
Pan-sensitizers
sgRNA:
DCK
C1orf109
MRPL20
BCAR1
XPO5
SKIV2L2
STK11
RNF149
SMARCB1
USP10
RYR3
MMS19
TRIM28
CHD7
ZBP1
ABCA13
ARID1A
MSN
KMT2C
NR5A2
MTA1
TERT
RBM10
UBR5
ZNRF3
EIF2D
BRCC3
BRD9
APOBEC3D
BRIP1
RNF113A
AsPC-1
HPAF-II
BxPC-3
Panc 10.05
Panc 08.13
HPDE
Response to
gemcitabine
Resistant
No effect
Sensitive

Genome-wide CRISPR–Cas9 screens reveal modulators of gemcitabine sensitivity. Credit: *Nature Cancer* (2024). DOI: 10.1038/s43018-024-00742-z

Researchers at the University of Toronto's Donnelly Centre for Cellular and Biomolecular Research have found two enzymes that work against the chemotherapy drug gemcitabine, preventing it from effectively treating pancreatic cancer.

The enzymes—APOBEC3C and APOBEC3D—increase during gemcitabine treatment and promote resistance to DNA replication stress in pancreatic cancer cells.

This, in turn, counteracts the effects of gemcitabine and allows for the growth of cancer cells.

"Pancreatic cancer has proven to be very challenging to treat, as it is usually diagnosed at stage 3 or 4," said Tajinder Ubhi, first author on the study and a former Ph.D. student in biochemistry in U of T's Temerty Faculty of Medicine.

"It is the most lethal type of cancer in Canada, with an average survival time of less than two years. While chemotherapy with gemcitabine has increased survival by a few months in clinical trials, options for treatment of pancreatic cancer remain limited."

The findings were published in the journal *Nature Cancer*.

Replication stress is the key process by which gemcitabine stops cancer

cells from continuing to multiply. It involves the dysregulation of DNA replication, which occurs when cells divide. Replication stress can transform a healthy cell into a cancerous one, but can also be activated within cancer cells to eliminate them.

Gemcitabine has been used for nearly three decades to treat a wide variety of cancers, including pancreatic, breast and bladder cancer. However, a downside of using gemcitabine to target dividing cells is that it can produce toxic side effects in tissues that aren't being targeted for treatment.

Ubhi and other members of Professor Grant Brown's lab at the Donnelly Centre have been trying to understand the possible causes of replication stress and its impacts. One way to do this is by studying the stress response mechanisms in cancer cells treated with gemcitabine.

"We conducted a genome-wide CRISPR screen to find genes that could increase the sensitivity of pancreatic cancer cells to gemcitabine," said Brown, professor of biochemistry at the Donnelly Centre and in the Temerty Faculty of Medicine, who is the principal investigator on the study.

"We were excited to identify APOBEC3C and APOBEC3D because other enzymes in the APOBEC3 family can cause cancers to eventually become resistant to treatment. We discovered a more direct role for the enzymes, where they actually protect pancreatic cancer cells from gemcitabine therapy."

Neither enzyme is naturally found in high concentrations within healthy or cancerous cells. The catch is that the replication stress the drug causes in pancreatic cancer cells in turn triggers an increase in both enzymes. The research team found that removing either APOBEC3C or APOBEC3D kills pancreatic cells by stymieing DNA repair and

destabilizing the cell genome.

"What is most exciting is that the removal of just APOBEC3C or APOBEC3D is enough to stop the replication of gemcitabine-treated pancreatic cancer cells," said Ubhi. "This indicates that the enzymes could be effective new targets for treating this form of cancer."

More information: Tajinder Ubhi et al, Cytidine deaminases APOBEC3C and APOBEC3D promote DNA replication stress resistance in pancreatic cancer cells, *Nature Cancer* (2024). DOI: 10.1038/s43018-024-00742-z

Provided by University of Toronto