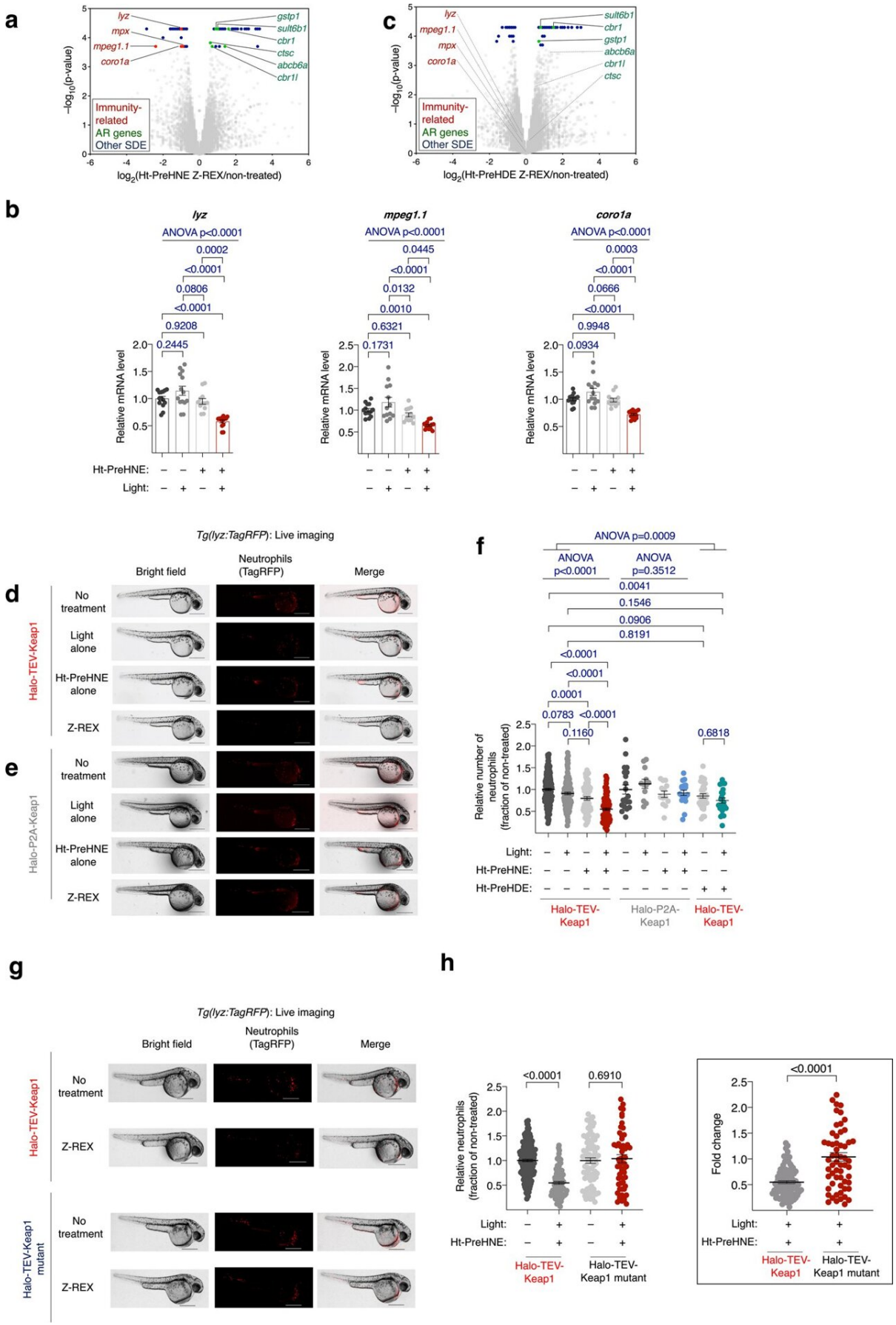
A tool to interrogate a new class of drugs

September 30 2021


a
lyz
mpx
mpeg1.1
coro1a
gstp1
sult6b1
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abcb6a
cbr1l
Immunity-related
AR genes
Other SDE
−log10(p-value)
log2(Ht-PreHNE Z-REX/non-treated)
c
lyz
mpeg1.1
mpx
coro1a
sult6b1
cbr1
gstp1
abcb6a
cbr1l
ctsc
Immunity-related
AR genes
Other SDE
−log10(p-value)
log2(Ht-PreHDE Z-REX/non-treated)
b
lyz
ANOVA p<0.0001
0.0002
<0.0001
0.0806
<0.0001
0.9208
0.2445
Relative mRNA level
Ht-PreHNE:
Light:
mpeg1.1
ANOVA p<0.0001
0.0445
<0.0001
0.0132
0.0010
0.6321
0.1731
Relative mRNA level
coro1a
ANOVA p<0.0001
0.0003
<0.0001
0.0666
<0.0001
0.9948
0.0934
Relative mRNA level
Tg(lyz:TagRFP): Live imaging
Bright field
Neutrophils (TagRFP)
Merge
d
Halo-TEV-Keap1
No treatment
Light alone
Ht-PreHNE alone
Z-REX
e
Halo-P2A-Keap1
No treatment
Light alone
Ht-PreHNE alone
Z-REX
f
ANOVA p=0.0009
ANOVA p<0.0001
ANOVA p=0.3512
0.0041
0.1546
0.0906
0.8191
<0.0001
<0.0001
0.0001
0.0783
<0.0001
0.1160
0.6818
Relative number of neutrophils (fraction of non-treated)
Light:
Ht-PreHNE:
Ht-PreHDE:
Halo-TEV-Keap1
Halo-P2A-Keap1
Halo-TEV-Keap1
g
Tg(lyz:TagRFP): Live imaging
Bright field
Neutrophils (TagRFP)
Merge
Halo-TEV-Keap1
No treatment
Z-REX
Halo-TEV-Keap1 mutant
No treatment
Z-REX
h
<0.0001
0.6910
Relative neutrophils (fraction of non-treated)
Light:
Ht-PreHNE:
Halo-TEV-Keap1
Halo-TEV-Keap1 mutant
<0.0001
Fold change
Light:
Ht-PreHNE:
Halo-TEV-Keap1
Halo-TEV-Keap1 mutant

Fig. 1: Immunity-related genes and neutrophils are suppressed in zebrafish embryos following Keap1-specific hydroxynonenylation. a Differential expression from RNA sequencing of embryos subjected to Keap1-hydroxynonenylation by Z-REX. Statistically-significant differentially-expressed (SDE) Nrf2-driven AR genes marked with green dots; SDE immunity-related genes with red dots; all other SDE genes with blue dots; and non-SDE genes with gray dots. b qRT-PCR analysis validated suppression of immunity-related genes in zebrafish embryos following Z-REX-assisted Keap1-specific hydroxynonenylation. P values were calculated with ANOVA and Tukey's multiple comparisons test. c Same as in (a) except Z-REX-mediated modification of Keap1 was executed with a different electrophile, HDE, of similar Keap1-modification efficiency53 and ability to upregulate AR genes [green dots]. Gray dashed lines mark genes not SDE in this comparison. See also Supplementary Table 1 and Supplementary Data 1. d Z-REX-mediated Keap1-hydroxynonenylation, but not Z-REX-technical controls (Supplementary Fig. 1a), caused depletion of neutrophil count in Tg(lyz:TagRFP), in which neutrophils are labeled with TagRFP. Scale bars, 500 μm. e Same as in (d) in fish expressing Halo-P2A-Keap1, which cannot undergo Keap1-hydroxynonenylation by Z-REX (Supplementary Fig. 1a). Scale bars, 500 μm. f Quantitation of neutrophil levels in Tg(lyz:TagRFP) following Z-REX (against all technical controls) using photocaged Z-REX probes, Ht-PreH(D)NE, delivering HN(D)E. Note: signal-to-noise in these experiments (d–f) is 6:1, so gene expression changes rendering signal below detection levels cannot explain this loss of neutrophils. Tukey's multiple comparisons test was used to calculate corrected p values. g Similar experiment as in (d) in fish expressing either Halo-TEV-Keap1 or Halo-TEV-Keap1C151S&C273W&C288E ('Halo-TEV-Keap1 mutant') which cannot undergo Keap1-hydroxynonenylation. Scale bars, 500 μm. h Quantitation of neutrophil levels in Tg(lyz:TagRFP) following Z-REX using photocaged Z-REX probes, Ht-PreHNE, delivering HNE (d and g). Inset: analysis of fold change (Z-REX/non-treated; see Fig. 2d) in neutrophil count. Note: for Halo-TEV-Keap1 set, the same data are presented in panels (f) and (h) for clarity. P values were calculated with two-tailed Student's t-test. All data present mean ± SEM. All p values for differential expression in RNA-seq were calculated with CuffDiff. All sample sizes are listed in Supplementary Methods.

Source data are provided as a Source data file. Credit: DOI: 10.1038/s41467-021-25466-x

In 2014, the European Medicines Agency approved the drug Tecfidera for the treatment of relapsing multiple sclerosis, a neurodegenerative disease that affects millions of people worldwide. In multiple sclerosis, inflammation damages the protective myelin insulation around nerves, and the nerves themselves. The active ingredient of Tecfidera is dimethyl fumarate, a compound that is thought to modulate the immune system, thus acting as an anti-inflammatory that alleviates the symptoms of multiple sclerosis.

But there was a detail of Tecfidera's approval that might have been a little less appreciated: it brought into the market a member of the relatively new—and still largely unexplored—class of drugs known as reactive electrophiles.

Reactive electrophilic compounds like dimethyl fumarate are molecules that "seek" to bond with atoms or other molecules that have an available electron pair. Adding an electrophilic unit to certain drugs significantly increases pharmacological efficacy, which has generated a lot of research activity into this area.

The problem, however, is that we don't know exactly how most reactive electrophilic drugs work, which makes it difficult to predict their effects and outcomes, and efficiently design new ones. The main obstacle is that reactive electrophiles seem to be very "promiscuous" inside the body or even a cell, bonding with multiple targets aside from the ones intended, which can result in unexpected side-effects and drug toxicity, and, in extreme cases, death.

Now, a team of scientists at EPFL led by Professor Yimon Aye, have been able to make a significant breakthrough in studying the effects of reactive electrophiles in the body. The scientists used a technique called "targetable reactive electrophiles and oxidants" or T-REX for short. T-REX and the broader "REX technologies" were developed by Prof. Aye during her work at Cornell University as she sought to understand the mechanisms of electrophile signaling. First published in 2016, the T-REX method releases a specific electrophile to a target protein, the ramifications of which can be observed in space and time, and in live cells.

In this study, the researchers adapted T-REX to be compatible with zebrafish (a technique they named Z-REX), and used it to systematically investigate the interactions of the electrophilic dimethyl fumarate in Tecfidera, and how those interactions produce the immunomodulating effects of Tecfidera.

The scientists targeted the protein Keap-1, a known cancer and metastasis suppressor, which has been debated as a potential target for dimethyl fumarate. Using Z-REX to target Keap-1 with various electrophiles, they discovered that some of them triggered a signaling pathway that leads results to the apoptosis of neutrophils and macrophages.

That pathway also involves some novel "protein players" that had not been considered before in the Tecfidera field. By removing these "players," the researchers found that the anti-inflammatory effects of Tecfidera, which are what make it a treatment for multiple sclerosis, were abolished as well.

The work demonstrates that Z-REX, and by extension, the REX technologies, are effective tools for investigating the interactions of electrophilic compounds and drugs in living organisms.

More information: Jesse R. Poganik, Kuan-Ting Huang, Saba Parvez, Yi Zhao, Sruthi Raja, Marcus J. C. Long, Yimon Aye, Wdr1 and Cofilin are Necessary Mediators of Immune-Cell-Specific Apoptosis Triggered by Tecfidera. *Nature Communications* 30 September 2021. DOI: 10.1038/s41467-021-25466-x