Gene regulation: Silencing factor for endogenous retroviruses identified

October 19 2021

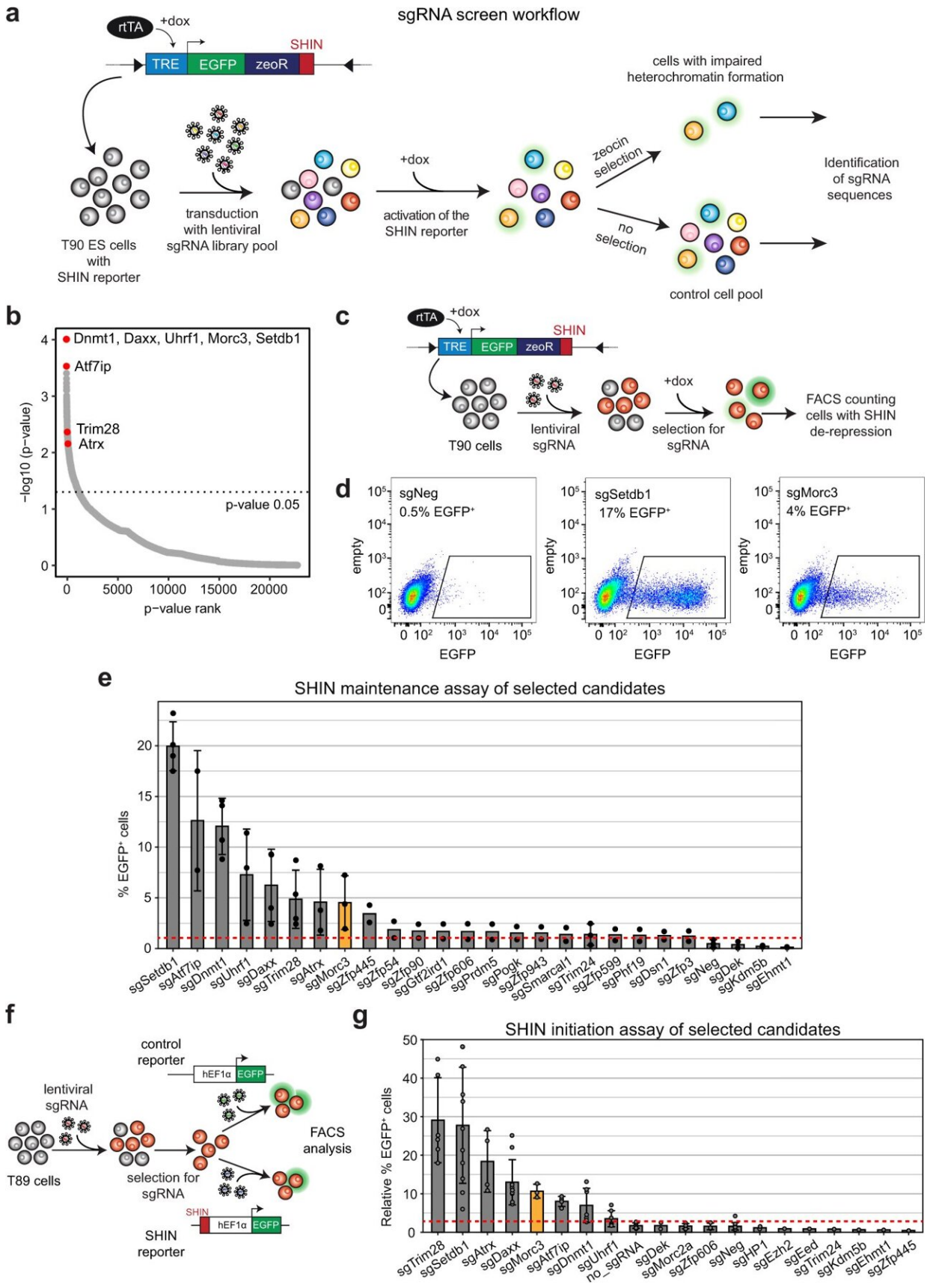
a
sgRNA screen workflow
rtTA
+dox
SHIN
TRE
EGFP
zeoR
T90 ES cells with SHIN reporter
transduction with lentiviral sgRNA library pool
+dox
activation of the SHIN reporter
cells with impaired heterochromatin formation
zeocin selection
no selection
Identification of sgRNA sequences
control cell pool
b
Dnmt1, Daxx, Uhrf1, Morc3, Setdb1
Atf7ip
Trim28
Atrx
p-value 0.05
−log10 (p−value)
p−value rank
c
rtTA
+dox
SHIN
TRE
EGFP
zeoR
T90 cells
lentiviral sgRNA
+dox
selection for sgRNA
FACS counting cells with SHIN de-repression
d
sgNeg
0.5% EGFP+
sgSetdb1
17% EGFP+
sgMorc3
4% EGFP+
empty
EGFP
e
SHIN maintenance assay of selected candidates
% EGFP+ cells
sgSetdb1
sgAtf7ip
sgDnmt1
sgUhrf1
sgDaxx
sgTrim28
sgAtrx
sgMorc3
sgZfp445
sgZfp54
sgZfp90
sgGtf2ird1
sgZfp606
sgPrdm5
sgPogk
sgZfp943
sgSmarcal1
sgTrim24
sgZfp599
sgPhf19
sgDsn1
sgZfp3
sgNeg
sgDek
sgKdm5b
sgEhmt1
f
control reporter
hEF1α EGFP
lentiviral sgRNA
T89 cells
selection for sgRNA
FACS analysis
SHIN
hEF1α EGFP
SHIN reporter
g
SHIN initiation assay of selected candidates
Relative % EGFP+ cells
sgTrim28
sgSetdb1
sgAtrx
sgDaxx
sgMorc3
sgAtf7ip
sgDnmt1
sgUhrf1
no_sgRNA
sgDek
sgMorc2a
sgZfp606
sgNeg
sgHP1
sgEzh2
sgEed
sgTrim24
sgKdm5b
sgEhmt1
sgZfp445

Fig. 1: A genome-wide sgRNA screen for SHIN silencing identifies Morc3. **a** Setup of the sgRNA screen. T90 ES cells containing an inducible SHIN reporter20 were transduced with a genome-wide sgRNA library pool. Cells were selected for sgRNA vectors and the reporter was activated with doxycycline. Two independent cell batches were cultured to harvest non-selected cells representing the control, and zeocin-resistant cells representing cells with impaired heterochromatin on the SHIN reporter. **b** Dot plot showing the sgRNA screen results ordered by p-value rank (RIGER SecondBestRank scoring). Major ERV silencing factors are indicated. Morc3 represents a top hit in the screen. **c** Schematic of the SHIN silencing maintenance assay. T90 ES cells were transduced with sgRNAs for selected candidates and treated for integration with puromycin. Subsequently, doxycycline was added to the cells to induce reporter activity. EGFP expression was analyzed by FACS. **d** FACS plots depicting EGFP fluorescence in cells with activated SHIN reporter. Almost no activity was detected in cells transduced with a control sgRNA, demonstrating full SHIN silencing. sgRNAs targeting Setdb1 or Morc3 result in SHIN de-repression as indicated by cells showing EGFP expression. **e** Bar plot depicting the results of the SHIN maintenance silencing assay with selected candidate genes. The red dotted line indicates the background reporter activity. Data are presented as mean values +/− SD of biological replicates per sgRNA (n = 2–4, for details see "Statistics and reproducibility" section). **f** Schematic of the SHIN silencing initiation assay. T89 ES cells were transduced with sgRNAs for selected candidates and treated for integration with puromycin. Subsequently, the cells were transduced with a control virus without the SHIN sequence or the SHIN reporter with a constitutive promoter, respectively. EGFP expression was analyzed by FACS and the percentage of EGFP expressing cells, relative to the control reporter was calculated. **g** Bar plot depicting the results of the SHIN initiation silencing assay with selected candidate genes. The red dotted line indicates the background reporter activity. Data are presented as mean +/− SD of biological replicates per sgRNA (n = 1–12, for details see "Statistics and reproducibility" section). Credit: DOI: 10.1038/s41467-021-26288-7

Over time, many endogenous retrovirus (ERV) sequences have

integrated into the human genome, and now play important roles in normal gene regulation.

However, high levels of expression of ERV transcripts can lead to disorders such as autoimmune diseases and cancers. Based on a genome-wide screen, a team led by Gunnar Schotta at LMU's Biomedical Center has identified the protein Morc3 as a new factor involved in the silencing of ERVs.

Whether a given gene is expressed is largely determined by how its DNA sequence is packaged by histone proteins in the complex known as chromatin.

The new study shows that Morc3 triggers a modification of packaging by enabling the localized incorporation of the histone variant H3.3 into chromatin, which is mediated by the protein Daxx.

This modification results in the formation of a condensed chromatin structure that inhibits the activation of ERV genes.

The study is published in *Nature Communications*.

More information: Sophia Groh et al, Morc3 silences endogenous retroviruses by enabling Daxx-mediated histone H3.3 incorporation, *Nature Communications* (2021). DOI: 10.1038/s41467-021-26288-7

Provided by Ludwig Maximilian University of Munich

Citation: Gene regulation: Silencing factor for endogenous retroviruses identified (2021, October 19) retrieved 19 April 2024 from https://phys.org/news/2021-10-gene-silencing-factor-endogenous-retroviruses.html

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