

Core RNA Polymerase II TOR

Ma ^{1,2} Aa ^{1,2} Ag ^{1,2} Ma ^{1,2} Pa ^{1,2} Aa ^{1,2}
To ^{1,2} Ca ^{1,2} Ma ^{1,2} Ka ^{1,2} Da ^{1,2} Fa ^{1,2} Ka ^{1,2} Go ^{1,2} Da ^{1,2}
Na ^{1,2,8} Ma ^{1,2,8} Jo ^{1,2,8} Ka ^{1,2,8} Ta ^{1,2,8} Ca ^{1,2,8} Ma ^{1,2,8} Go ^{1,2,8}

¹MRC (MRC) LMS (LMS), L W12 ONN, U K ; ²ICS (ICS), F M Ib C L W12 ONN, U K ; ³MRC R M U E EH16 4UU, U K ; ⁴W G B L-1526 L L P T B C CB22 3AT, U K ; ⁵R M P (IMP), V B (VBC), V 1030, A ; ⁶MRC H G U ; ⁷E E EH4 2XU, U K

ES cell pluripotency is maintained by OCT4, SOX2, KLF4, and MYC (OSKM) factors. These factors are essential for the establishment and maintenance of pluripotency. The TOR pathway is a central regulator of cell growth and metabolism, and its inhibition is known to enhance pluripotency. In this study, we have investigated the role of the TOR pathway in the establishment and maintenance of pluripotency. We have found that inhibition of TOR activity by rapamycin (rapamycin) significantly enhances the efficiency of ES cell reprogramming. This effect is dependent on the presence of OSKM factors. Our results suggest that the TOR pathway acts as a negative regulator of pluripotency, and its inhibition may be a useful strategy for enhancing the efficiency of ES cell reprogramming.

[K : SASP; ; PSC ; RNA; ; RNA.]

Sub
R F 20, 2017; O 18, 2017.

D (OCT4, SOX2, KLF4, MYC, OSKM) ES T (G 1962; W 1997) (ES) (T 2001). I Y (T Y 2006)

(Q 2014; S 2014; Y 2014; C 2015). A 2010). T (K 2010). TERT (Q 2014), PSC (K L 2009; B G 2010). K 53, 16^{INK4}, 21^{CIP1} OSKM, (B 2009; H 2009; K 2009; L 2009; M 2009; U 2009). H S Ab (SASP) (K 2010; S 2014). S SASP (C 2010). S (M 2014), (B 2016), 2008) (C S 2010). S (M 2016). T IL-6 SASP, IL-6 MYC PIM1 (B 2013). G G (J 2000; R 2005; A 2008; T 2016; W 2016) (Q 2014; S 2014; Y 2014). I RNA OSKM RNA T RNA35-226 RNA U RNA TOR 3528 3531 3512 a a 53,352 M RNA S a

E a OSKM (OSKM) IMR90 (F 1A,B) (B 2009). S RAS^{G12V} OSKM (CDK) (CDKI) 15^{INK4} 16^{INK4}, 21^{CIP1} (F 1C). T OSKM-RNA (RNA:) G (GSEA) SASP OSKM (F 1D). O RAS- F TGF- β (SASP) F S1A). I RAS OSKM F RAS OSKM (SASP) F S1B), RAS (F 1E). O RAS OSKM (=0.33) (F 1F). A (F 1G; SASP) F S1C), (GO) (F 1H; SASP) F S1D). B OSKM RAS GO OSKM RAS (F 1I; SASP) F S1E). O OSKM

a 3 35223 -118a -2 a 12 12 a

352218 -236 -2283 -33 RNc T/T -36 -8 -383

Fig. 1. OSKM IMR90 (A) S IMR90 OSKM RAS. A 12 () () (SA-β-G) () B U
 18- () B, 100 M. (B) Q B U SA-β-G
 (***) $P < 0.001$. (C) Q RT-PCR (RT-PCR). RNA () *CDKN2B* ()
 15^{INK4}), *CDKN2A* (16^{INK4}), *CDKN1A* (21^{CIP1}) () (*) $P < 0.05$; (***) $P < 0.001$. (D) G
 (GSEA). SASP OSKM
 IMR90 (NES) N (E) H (C 2004).
 IMR90 RAS OSKM. L 2 () Z;
 RAS OSKM (FC) B
 (F) S (FC) RAS OSKM
 [FDR] < 0.05; < -1 > 1 (G) V
 RAS OSKM D
 < -1 FDR < 0.05. (H,I) G (GO)
 OSKM- RAS- (H) OSKM- (I). F. N
 M (//) GO S.
 O (P < 0.05)

(37 ... 0) (F 2B,C). A RNA
 S2A. A. RNA RNA
 RNA
 ; 3153. RNA (*CDKN1A*, *MTOR*, *MYOT*, *UBE2E1*)
 (F 2A). S RNA (F 2D);
 OSKM- RNA
 S2D).

F 2. A RNA. IMR90. OSKM (A) T RNA

T OSKM IMR90 RNA
 RNA W (S3A-C). MYOT RNA
 RNA T RNA
 CDKN1A, MYOT, MTOR, UBE2E1
 OSKM- (F . 2E), B U (F . 2F; S3B) F . S3D),
 (SA-β-Gal) IMR90 OSKM
 (F . 2G,H; S3C) F . S3E). S 21^{CIP1}
 (B, 2009),

3E), TP53, CDKN1A, MTOR, UBE2E1
 RNA (F . 3F). M
 CDKN1A, 53 OSKM- 53 W MYOT
 F RNA
 (F . 3G; S3B) F . S4C). T RNA
 W (.06 19.6720T 19.7353.6)

RNA- a a a a a a a a
 RNA

A RNA- T
 (T R 2017).
 W RNA RNA

T RNA (F . 3A). T RNA
 R-E, R-30-
 (F 2013),
 RNA II(P II)- RNA (D
 2005). W RNA
 -A). 3'
 (LTR) -A. (ATTAAA) 3' R-E,
 T RNA,
 RNA R-E (S3B)
 F . S4A). T RNA (S3B) F . S4B)
 RNA (F . 3B).
 M RNA (116 300)
 (F . 3C), RNA (F . 3D).
 (310 359. RNA)
 N RNA: IMR90
 OSKM RNA (F .
 3E). C RNA
 RNA (RNA
 MTOR, CDKN1A MYOT
 UBE2E1, T RNA:
 RNA (F .

F 3. C RNA RNA (A) S
RNA IMR90 OSKM RNA RNA ICCELL8
(B) T RNA IMR90 RNA RNA
; = 50, OSKM RNA (OSKM/L ;

TGF- β a 21^{CIP1} OSKM-

T MTOR OSKM-
RAS- W MTOR OSKM-

OSKM- (F. 5A). T
RAS- OSKM- (H. 2015). I

F 5. TOR OSKM OSKM (A) K MTOR RNA
 TOR OSKM () RAS () IMR90 (B) I
 OSKM RAS 0.3 1.0 M (B) A 12 (C,D) I
 TOR CDKI OSKM IMR90
 OSKM RAS RT-PCR RNA DMSO (-) (C)
 A 10

OSKM RAS RNA
 (S6A) S6A) B RNA
 CDKN2A, CDKN2B,
 CDKN1A TOR
 OSKM (RAS)

C RNA, CDKN2B
 OSKM, TOR (F 5C) A
 CDKN2A CDKN1A,
 RAS A
 CDKI

CDKI OSKM RAS
(F . 5C). A 16^{INK4} W.
16^{INK4} OSKM-
RAS- (F . 5D; S6B).
W
CDKN1A, CDKN2A, CDKN2B OSKM-
D CDKN2B
(F . 4E, 5C), CDKN2B
RNA
OSKM (F . 5E; S6C;
). O CDKN1A

Aa4c4 a .

F 6. D TOR
PSC (A) Rb
C. 9- b TNG MKOS MEF
1
Ba MKOS
RNA RNA
N -GFP+
14. S Sbb F S7, B
C. SD E
SD
(*) P < 0.05; (**) P < 0.01;
(***) P < 0.001; () (B) D
(0.3 1.0
M) 3, 6, 14 MKOS
R PSC
(AP)
14 D SD
E SD
(0-3 0-6).
D 0-14. (*) P < 0.05; (**) P < 0.01; (***) P <
0.001. (C) D (0.3
1.0 M) 3 MKOS
R PSC
14 (D) R
C. 9- b N
GFP MEF
Ba
MKOS O U6 RNA
500 M TGF-β RI (A 5 ;
T A83-01) 5 M (B)
N N -GFP+
14 E
SD (*)

P < 0.05; (**) P < 0.01; () (G) S N + ; () . (E) Rb
MKOS MEF (CM) MEF RAS,
RAS RNA M CM 3 4
MEF A (AP+)
CM. RAS/ E SD (**) P < 0.01; (***) P < 0.001. Rb
(F) S MTOR PSC (G)

S7F). O TOR F 6F).
TOR F 6F).

D c o
A
M
(Q 2014; S 2014; Y 2014). S
2009). D (B,
2009). D

(K L 2009; B. 53 16^{INK4}, 21^{CIP1}
G 2010).
RNA
OSKM- O
OSKM- W
RNA : CDKN1A, MTOR,
MYOT, UBE2E1. W CDKN1A (
21^{CIP1}) MTOR
MYOT, UBE2E1
MYOT Z
(O 2005). D
RNA MYOT
MYOT RNA
MYOT IMR90 UBE2E1

E2 (N 1996).
P - b (D -S
2013). W UBE2E b
G b 21^{CIP1}

Pa

F RE- RNA, 97-
RNA PCR-
RE-X RE-E O
RLL (F
2013).. RNA. S T S1.

RNA a a

D RNA
M

L a a a RNA

G DNA 10⁶ G P
(Q S
(3 [] GIPZ 4
RLL), PCR 2 μ
DNA I PCR
QIA (Q
Q 2.0 F
(40). P
S PCR
B A DNA (A T
) O 12
M G A II H S
2500 (50-),

S a a a a RNA

FASTA. CASAVA 1.8.
T RNA B (0.12.8). C
F P- R. T F
P- (F
1925).

I a a

C 4% 30
PBS, 0.2% T X-100. 5

References

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P S, F, OR, B AK, W, G, S, S,
S, R. 2014. F RNA:
S : 2. *Na P* 9: 171-181.

Coupling shRNA screens with single-cell RNA-seq identifies a dual role for mTOR in reprogramming-induced senescence

Marieke Aarts, Athena Georgilis, Meryam Beniazza, et al.

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