Science Signaling
AAAS
www.sciencesignaling.org/cgi/content/full/3/130/ra52/DC1

Supplementary Materials for

Regulation of Notch1 Signaling by Nrf2: Implications for Tissue Regeneration

Nobunao Wakabayashi,* Soona Shin, Stephen L. Slocum, Elin S. Agoston, Junko Wakabayashi, Mi-Kyoung Kwak, Vikas Misra, Shyam Biswal, Masayuki Yamamoto, Thomas W. Kensler

*To whom correspondence should be addressed. E-mail: nw99@pitt.edu

Published 20 July 2010, *Sci. Signal.* **3**, ra52 (2010)
DOI: 10.1126/scisignal.2000762

The PDF file includes:

Fig. S1. Differential expression of *Notch1* in *Keap1-Nrf2* compound knockout MEFs and livers.
Fig. S2. *Nqo1* and *Hes1* gene expression after partial hepatectomies.
Table S1. Oligonucleotides used in experiments.
Table S2. PCR conditions for genotyping.
References

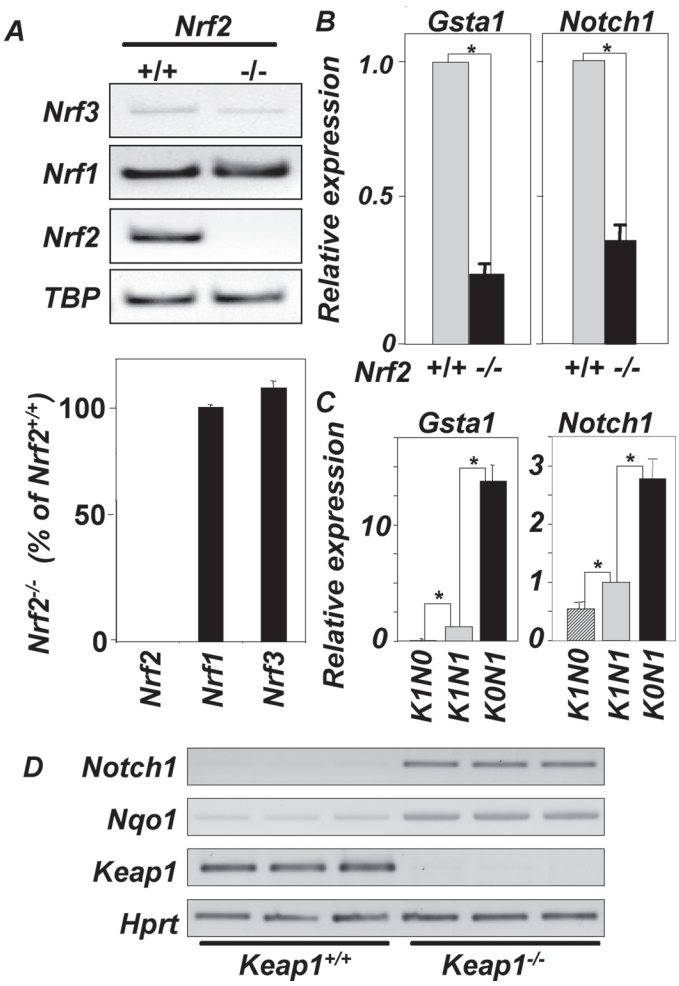

Fig. S1. Differential expression of *Notch1* in *Keap1-Nrf2* compound knockout MEFs and livers. (**A**) Semi-quantitative RT- PCR for *Nrf1* and *Nrf3* genes in MEFs. The abundance of the transcript for Tata binding protein (TBP) served as a loading control. Data are quantified as the ratio of the expression of the indicated genes in *Nrf2* null/wild type. Values are the means of 3 independent experiments ± S.E. Expression of both genes was not altered between Nrf2 genotypes in MEFs (t- test). (**B**) Real time RT-PCR analyses of *Notch1* and *Gsta1* expression in the livers of 8-week old *Nrf2*-null and wild-type mice. n=3, *p<0.01 (**C**) Real time RT- PCR analyses of *Notch1* and *Gsta1* expression in the livers of various 8-week old *Keap1* and *Nrf2* compound-disrupted mice. n=3, *p<0.01. K0N1, K1N1 and K1N0 indicate *Keap1*$^{-/-}$::*Nrf2*$^{+/-}$, *Keap1*$^{+/-}$::*Nrf2*$^{+/-}$, and *Keap1*$^{+/-}$::*Nrf2*$^{-/-}$mice, respectively. (C) RT- PCR analyses of *Notch1* and *Nqo1* expression in the livers of postnatal day 1 *Keap1*$^{-/-}$ mice. *Hprt* served as the loading control.

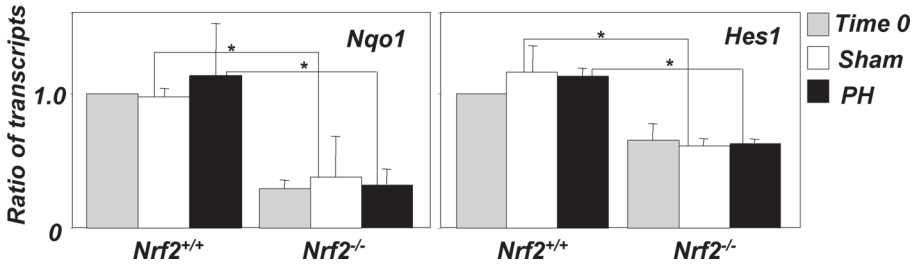

Fig. S2. *Nqo1* and *Hes1* gene expression after partial hepatectomies. *Hes1* and *Nqo1* expression were monitored by real-time RT-PCR as an index of Notch1 signaling 0.5 hr after 2/3 partial hepatectomy. *Albumin* expression was used for normalization. The ratio of the hepatic gene of interest to *albumin* expression in $Nrf2^{+/+}$ mice at time 0 was set at a value of 1.0. Values are mean ± S.D., n= 3, * $p < 0.05$, one-way analysis of variance.

TABLE S1. Oligonucleotides used in experiments.

RT-PCR experiment

Gene	Forward	Reverse	References
Nfe2l2	*CCATTTACGGAGACCCACCGCCTG*	*CTCGTGTGAGATGAGCCTCTAAGCGG*	*original, 273 bp product*
Nfe2l1	*GCAGTGCCTAGTGAGAGTGAG*	*ACGCCTGATGCAGGCTGACATTC*	*original, 255 bp product*
Nfe2l3	*GGAGCCAGGAGAACGGGATGCTAAGAG*	*GAGTGGCTTGCTCAGGATTTGTATGAG*	*original, 501 bp product*
Notch1	*CCTCATGATTGCCTCCTGCAGTGG*	*CAGGATCAGTGGAGTTGTGCCATCATGCA*	*(1)*
Notch1-2	*GGCACTGTGGATTATGCCTG*	*AGCGACAGATGTATGAAGACTCA*	PrimerBank ID 31339069a2
Rbpj	*GACTCACTAGGGAAGCTATGCG*	*TCCATCTCGTTCCATTTGCTC*	PrimerBank ID 30411012a2
p21	*CCGAGAACGGTGGAACTTTGACT*	*GCAGAAGACCAATCTGCGCTTGG*	*(2)*
Hes1	*TCAACACGACACCGGACAAACC*	*GGTACTTCCCCAACACGCTCG*	*(3)*
Hes1-2	*ATAGCTCCCGGCATTCCAAG*	*GCGCGGTATTTCCCCAACA*	PrimerBank ID 6680205a2
Herp1	*CAATTCACCGACAACTACCTCTC*	*GCCTTTTCTAACTTGGCAGATCC*	PrimerBank ID 7305159a2
Herp2	*GCGCGGACGAGAATGGAAA*	*TCAGGTGATCCACAGTCATCTG*	PrimerBank ID 6754188a1
Nrarp	*AAGCTGTTGGTCAAGTTCGGA*	*CGCACACCGAGGTAGTTGG*	PrimerBank ID 16751909a1
Jag1	*CCAGCCAGTGAAGACCAAGT*	*TCAGCAGAGGAACCAGGAAA*	*(4)*
Dll1	*CTGAGGTGTAAGATGGAAGCG*	*CAACTGTCCATAGTGCAATGG*	*(5)*
Nqo1	*CATTCTGAAAGGCTGGTTTGA*	*TTTCTTCCATCCTTCCAGGAT*	*(6)*
Nqo1-2	*GGCATCCTGCGTTTCTGTG*	*GGTTTCCAGACGTTTCTTCCAT*	PrimerBank ID 6679080a3
Gsta1	*CCGTTACTTGCCTGCCTTTG*	*TGCAGCTTCACTGAATCTTGAAAG*	*(7)*
Gsta1-2	*CGCAGACCAGAGCCATTCTC*	*TTGCCCAATCATTTCAGTCAGAT*	*(8)*
Alb	*TGCTTTTTCCAGGGGTGTGTT*	*TTACTTCCTGCACTAATTTGGCA*	Primer Bank ID 33859506a1
Tbp	*AAGAGAGCCACGGACAACTG*	*TACTGAACTGCTGGTGGGTC*	*(4)*
Tbp-2	*TCTACCGTGAATCTTGGCTGT*	*CTGGCTCATAGCTCTTGGCTC*	*(8)*
Hprt	*GCTGGTGAAAAGGACCTCTC*	*CACAGGACTAGAACACCTGC*	*(9)*
18S	*GTAACCCGTTGAACCCCATT*	*CCATCCAATCGGTAGTAGCG*	*(10)*
Keap1	*CCCATG GAAAGGCTTATTGAGTTC*	*GAAGTGCATGTAGATATACTCCC*	*original, 230 bp product*

Primer Bank ; http://pga.mgh.harvard.edu/primerbank/

EMSA

Name	Oligonucleotides
Notch1-ARE	GGAGCGCCCGCCCGCTCAGAGCCTCAC
Notch1-mutARE	GGAGCGCCCGCCCGCAAAGAGCCTCAC
Gsta1-ARE	CTTGGAAATGACATTGCTAATGGTG

-206 reporter serial mutant constructs for PCR cloning

Construct	Oligonucleotides
-206 wild-type	CGGTACCGCCCGCTCAGAGCCTCACTAGTGCCTCTGCCGCGG
-206AREmut	CGGTACCGCCCGCAAAGAGCCTCACTAGTGCCTCTGCCGCGG
Notch1 3-2	CGTGCCATGGCTGCTCGCCAGCTGCCCGCAGCCCGGGCGGCG

TABLE S2. PCR conditions for genotyping.

Genotyping for RCKF cells

5-cko 4int1	GCACATCCTTCATCTCTCCGCACTGGGGAG	
3-Kp1-4Ex	CCTCCGTGTCAACATTGGCGCGACTAG	
R379-EGFP	TCAGCTCGATGCGGTTCACC	
Cre1	ACGTTCACCGGCATCAACGT	for *CreER*
Cre2	CTGCATTACCGGTCGATGCA	
IMR3621	CGTGATCTGCAACTCCAGTC	http://jaxmice.jax.org/support/genotyping/index.html
IMR3622	GGAGCGGGAGAAATGGATATG	for wild-type *Rosa* locus

Reaction for *Keap1*^{flox}MEF

Step #	Temp °C	Time	Note
1	95	1 min	-
2	95	30 sec	-
3	68.5	30 sec	-
4	72	30 sec	repeat steps 2-4 for 35 cycles
5	72	1 min	-
6	4	-	hold

Product Size ----------Undisrupted : ~350 bp, Disrupted : ~650 bp, Wt : ~250 bp

Reaction for *Cre* transgenic allele

Step #	Temp °C	Time	Note
1	94	1 min	-
2	94	30 sec	-
3	60	30 sec	-
4	72	30 sec	repeat steps 2-4 for 35 cycles
5	4	-	hold

Product Size ----------*Cre*-Tg : 355 bp

Reaction for *Rosa Wt allele (*http://jaxmice.jax.org/support/genotyping/index.html)

Step #	Temp °C	Time	Note
1	94	3 min	-
2	94	30 sec	-
3	68	1 min	-
4	72	1 min	repeat steps 2-4 for 35 cycles
5	72	2 min	-
6	10	-	hold

Product Size ---------- Wt : 400 bp

Genotyping for *Nrf2*$^{-/-}$::*Rosa*$^{NICD/-}$::*AlbCre* mouse in Fig.5D.

IMR0883	AAAGTCGCTCTGAGTTGTTAT	for Rosa NICD
IMR8038	TAAGCCTGCCCAGAAGACTC	
IMR8039	GAAAGACCGCGAAGAGTTTG	
IMR0883	AAAGTCGCTCTGAGTTGTTAT	for Cre-active allele
PGK-3740	GATGTGGAATGTGTGCGAGGCCAGAGGC	
NICD-5477	GATTGTCGTCCATCAGAGCACCATCTGAGG	

Reaction for *Rosa NICD (*http://jaxmice.jax.org/support/genotyping/index.html)

Step #	Temp °C	Time	Note
1	94	3 min	-
2	94	30 sec	-
3	54	1 min	-
4	72	1 min	repeat steps 2-4 for 35 cycles
5	72	2 min	-
6	10	-	hold

Product Size ----------Tg : 320 bp, Wt : 235 bp

Reaction for Cre *active allele*

Step #	Temp °C	Time	Note
1	95	1 min	-
2	95	30 sec	-
3	65.4	30 sec	-
4	72	45 sec	repeat steps 2-4 for 35 cycles
5	72	2 min	-
6	4	-	hold

Product Size ----------Cre-Active : 650 bp, Cre-Inactive : 550 bp

References

1. X. Gao, W. Bian, J. Yang, K. Tang, H. Kitani, T. Atsumi, N. Jing, A role of N-cadherin in neuronal differentiation of embryonic carcinoma P19 cells, *Biochem Biophys. Res. Commun.* **284**, 1098-1103 (2001).

2. H. Fujii-Yamamoto, J. M. Kim, K. Arai, H. Masai, Cell cycle and developmental regulations of replication factors in mouse embryonic stem cells, *J. Biol. Chem.* **280**, 12976-12987 (2005).

3. J. Jensen, E. E. Pedersen, P. Galante, J. Hald, R. S. Heller, M. Ishibashi, R. Kageyama, F. Guillemot, P. Serup, O. D. Madsen, Control of endodermal endocrine development by Hes-1, *Nat. Genet.* **24**, 36-44 (2000).

4. S. Cormier, S. Vandormael-Pournin, C. Babinet, M. Cohen-Tannoudji, Developmental expression of the Notch signaling pathway genes during mouse preimplantation development, *Gene Expr. Patterns.* **4**, 713-717 (2004).

5. J. Fu, S. S. Tay, E. A. Ling, S. T. Dheen, High glucose alters the expression of genes involved in proliferation and cell-fate specification of embryonic neural stem cells, *Diabetologia.* **49**, 1027-1038 (2006).

6. D. A. Johnson, G. K. Andrews, W. Xu, J. A. Johnson, Activation of the antioxidant response element in primary cortical neuronal cultures derived from transgenic reporter mice, *J. Neurochem.* **81**, 1233-1241 (2002).

7. S. A. Chanas, Q. Jiang, M. McMahon, G. K. McWalter, L. I. McLellan, C. R. Elcombe, C. J. Henderson, C. R. Wolf, G. J. Moffat, K. Itoh, M. Yamamoto, J. D. Hayes, Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice, *Biochem. J.* **365**, 405-416 (2002).

8. S. Shin, N. Wakabayashi, V. Misra, S. Biswal, G. H. Lee, E. S. Agoston, M. Yamamoto, T. W. Kensler, NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis, *Mol. Cell. Biol.* **27**, 7188-7197 (2007).

9. T. Yamaguchi, P. Sharma, M. Athanasiou, A. Kumar, S. Yamada, M. R. Kuehn, Mutation of SENP1/SuPr-2 reveals an essential role for desumoylation in mouse development, *Mol. Cell. Biol.* **25**, 5171-5182 (2005).

10. T. D. Schmittgen, B. A. Zakrajsek, Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR, *J. Biochem. Biophys. Methods.* **46**, 69-81 (2000).