Redox signals at the ER-mitochondria interface control melanoma progression

Xin Zhang[1,2,*], Christine S. Gibhardt[1,*], Thorsten Will[3], Hedwig Stanisz[4], Christina Körbel[5], Miso Mitkovski[6], Ioana Stejerean[1], Sabrina Cappello[1], David Pacheu-Grau[7], Jan Dudek[7], Nasser Tahbaz[8], Lucas Mina[8], Thomas Simmen[8], Matthias W. Laschke[5], Michael D. Menger[5], Michael P. Schön[4], Volkhard Helms[3], Barbara A. Niemeyer[9], Peter Rehling[7,10], Adina Vultur[1], Ivan Bogeski[1,2,$]

[1]Molecular Physiology, Institute of Cardiovascular Physiology, University Medical Center, Georg-August-University, Göttingen, Germany

[2]Biophysics, Center for Integrative Physiology and Molecular Medicine, Saarland University, Homburg, Germany

[3]Center for Bioinformatics, Saarland University, Saarbrücken, Germany

[4]Department of Dermatology, Venereology and Allergology, University Medical Center, Georg-August-University, Göttingen, Germany

[5]Institute for Clinical and Experimental Surgery, Saarland University, Homburg, Germany

[6]Light Microscopy Facility, Max Planck Institute for Experimental Medicine, Göttingen, Germany

[7]Department of Cellular Biochemistry, University Medical Center, Germany

[8]Department of Cell Biology, University of Alberta, Edmonton, Canada

[9]Molecular Biophysics, Center for Integrative Physiology and Molecular Medicine, Saarland University, Homburg, Germany

[10]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

*equal contribution

[$]Correspondence:

Prof. Dr. med. Ivan Bogeski

Molecular Physiology

Institute of Cardiovascular Physiology, University Medical Center, Georg-August-University

Humboldtallee 23, 37073 Goettingen, Germany

Tel: 0551 39 65520, Fax: 0551 39 5895

E-Mail: ivan.bogeski@med.uni-goettingen.de

APPENDIX MATERIAL

Table S1: Panel of genetically distinct melanoma cell lines grouped for BRAF and N-RAS mutations

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APPENDIX TABLES

Table S1: Panel of genetically distinct melanoma cell lines grouped for BRAF and N-RAS mutations*

Cell line	BRAF	N-RAS
WM3734	V600E	WT
Mel Juso	WT	Q61K
WM164	V600E	WT
WM983B	V600E	WT
1205Lu	V600E	WT
WM9	V600E	WT
WM3918	WT	WT
SK Mel 5	V600E	WT
451Lu C2	V600E	WT
WM1366	WT	Q61L

All sequencing analyses were performed in the K. Nathanson laboratory, Cancer Genetics, University of Pennsylvania, Philadelphia, PA, USA). All cell lines have been fingerprinted for their uniqueness and to exclude cross-contamination. WT, wild type.

Table S2: Univariate Cox analysis

Descriptor	HR	CI 95 %-low	CI 95 %-high	z-score	p-value	LR-test p-value
TMX3 state	3.0853	1.4052	6.7738	2.8078	0.0050	0.0038
NFAT1 expr	1.0239	1.0049	1.0433	2.4726	0.0134	0.0578
NFAT1 state	1.8734	0.8897	3.9447	1.6523	0.0985	0.0987
TMX1 state	1.7227	0.8131	3.6497	1.4198	0.1557	0.1633
TMX3 expr	1.0786	0.9701	1.1993	1.3993	0.1617	0.1641
TMX1 expr	1.0169	0.9666	1.0697	0.6470	0.5176	0.5216

Table S3: Multivariate Cox analysis

Assessment of descriptors contributing to the model

Descriptor	HR	CI 95 %-low	CI 95 %-high	z-score	p-value (Pr(>|z|))
NFAT1 expr	1.02	1.001	1.039	2.053	0.04004
TMX3 state	2.943	1.331	6.508	2.666	0.00768

Assessment of the model (2 degrees of freedom)

Statistical test	p-value
Likelihood ratio test	0.003944
Wald test	0.001652
Log-rank test	0.000386

Table S4: **The role of BRAF genotype on NFAT1, TMX1 and TMX3 expression in melanoma patients***

	NFAT1		TMX1		TMX3	
	low	high	low	high	low	high
WT	34	15	38	11	30	19
V600E	22	26	28	20	25	23
Fisher's exact test	p=0.024		n.s		n.s	

Discretized mRNA expression of NFAT1, TMX1 and TMX3 in BRAF WT and BRAF V600E melanoma patients. The expression was characterized high or low as described in the manuscript.

Table S5: Differentially expressed genes after NFAT1 silencing in melanoma (data are extracted from (Shoshan et al., 2016) and (Aibar et al., 2017))

1) Mitochondrion-localized

2) Redox-related

3) Deregulating Cellular Energetics

4) Resisting Cell Death

5) Tumor-Promoting Inflammation

6) Genome Instability and Mutation

7) Evading Growth Suppressors

8) Avoiding Immune Destruction

9) Sustaining Proliferative Signaling

10) Activating Invasion and Metastasis

11) Enabling Replicative Immortality

12) Inducing Angiogenesis

Both (upregulated)

Gene	1)	2)	3)	4)	5)	6)	7)	8)	9)	10)	11)	12)
ACPP					X							
AMACR	X											
ARHGAP23												
BTBD1												
BTG2				X		X	X					
C15orf39												
CD276					X		X					
CDCP1												
CDK6					X		X		X			
CDKN1A				X			X				X	
CORO2B												
CSGALNACT1							X					
CTSA					X							
CYBRD1		X										

DHRS2	X	X		X	X						
DVL2						X		X			
FAM3C											
FAM46C											
FGFR1				X		X		X	X		X
GNPTAB											
HIPK3				X							
HSPA6					X						
HYOU1				X							
IL1A				X	X	X		X			
ITGA4					X			X	X		
JAG1								X			X
JAK1								X	X		
LAMB3									X		
LIF								X			
MAP1B											
MAP3K1				X				X			
MAP4K4				X							
MARCKSL1											
MKRN1											
NPL											
NPTX2											
NUMA1					X	X					
OLFML3											
PARP10											
PBXIP1											
PFKFB4											
PHLDA1				X		X					
PLEKHA4											
RAB5B					X		X				
RHOB				X		X			X		X
RNF19A											
RUSC2											
SMURF2								X			
SPP1					X				X		
SPRY1						X					
SYTL5											
TAP1	X						X				
TCN1					X						
TLN1								X	X		
TMEM8A											

Gene	1)	2)	3)	4)	5)	6)	7)	8)	9)	10)	11)	12)
TWF1												
UCN2												
USP22							X					
VAT1L		X										

Both (downregulated)

Gene	1)	2)	3)	4)	5)	6)	7)	8)	9)	10)	11)	12)
ACAN										X		
AIP									X			
AKR1C3		X		X								
ALDOC	X		X		X							
BCL7C				X								
BIK	X			X								
BLVRB		X										
BNIP3	X			X								
C1orf123												
CAPG												
CCDC12												
CCNB1	X					X	X					
CDCA4												
COPZ1												
COX6B1	X	X	X									
CSTB					X							
CUTA												
DEF8												
DPCD												
EIF4E							X					
ENPP2												
FAM162A	X			X								
FAT3										X		
GPI			X	X	X							X
ITIH5												
KLF9												
KRTCAP2												
LACTB2	X											
LARP6												
LCN2				X	X				X			
MDH2	X	X	X									
NDUFA11	X		X									
NDUFA7	X	X	X									
NDUFA9	X	X	X									

NDUFS3	X	X	X	X							
NNMT											
PAFAH1B3											
PDE6D											
PSENEN				X					X		
RABAC1											
RCN3											
RPL29											
RTN4IP1	X	X									
SH3BGRL3		X	X								
SLC25A1	X										
SMYD3											
SSU72											
STXBP6										X	
TFAP2B				X							
TMEM47											
TMSB10											
VAMP5											
VPS25											
ZDHHC4											
ZNF511											
ZNF706											

Table S6: Primers used for RT-qPCR (all indicated 5' to 3')

Transcript	Forward	Reverse
TMX1	AGTCCTGGTGCTGTTGCTTT	TTCTCCCCATTCAGCAAAAC
TMX3	TTGCTATGGATGGCTTCCTC	TGGGACTGTCAATTCATCCA
NFAT1	AAACTCGGCTCCAGAATCCA	TGGACTCTGGGATGTGAACT
NFAT2	GCTATGCATCCTCCAACGTC	AGTTGGACTCGTAGGAGGAG
NFAT3	ACACAGCCCTATCTTCAGGA	ATCTTGCCTGTGATACGGTG
NFAT4	ACCCTTTACCTGGAGCAAAC	CTTGCAGTAGCGACTGTCTT
NFAT5	CGTGTGTGTGGCTTCTATGT	TGCCTCTCAATCAGAGAGAG
XBP1	CACCTGAGCCCCGAGGAG	TTAGTTCATTAATGGCTTCCAGC
TBP	CGGAGAGTTCTGGGATTGT	GGTTCGTGGCTCTCTTATC
MCUa	CACACAGTTTGGCATTTTGG	TGTCTGTCTCTGGCTTCTGG
MCUb	TTTTGCGTGTGAAGCTGTGT	TACCAAGGGAAGGCCATGT
NCLX	ATGGTGGCTGTGTTCCTGACCT	GGTGCAGAGAATCACAGTGACC

Table S7: Primary antibodies used for immunoblotting and immunohistochemistry

TMX1	Goat pAb; TXNDC; Abcam #ab37876	1:1000 for immunoblotting 1:750 for immunohistochemistry
NFAT1	Rabbit mAb; NFAT1 (D43B1) XP; Cell Signal. Tech. #5861S	1:1000 for immunoblotting 1:750 for immunohistochemistry
HSP90	Rabbit Ab; HSP90; Cell Signal. Tech. #4874S	1:1000 for immunoblotting
Calnexin	Rabbit pAb; Calnexin; ENZO#ADI-SPA-860-F	1:1000 for immunoblotting
Actin	Mouse Ab; beta-actin; Sigma #A5441	1:10 000 for immunoblotting
AKT	Rabbit Ab; AKT (D9E); Cell Signal. Tech. #9272S	1:1000 for immunoblotting
p-AKT	Rabbit mAb; pAKT S473 (D9E); Cell Signal. Tech. #4060S	1:1000 for immunoblotting
GAPDH	Rabbit mAb; GAPDH (14C10); Cell Signal. Tech. #2118L	1:1000 for immunoblotting
BiP and PDI	antibodies raised in rabbit were kindly provided by Prof. Dr. Richard Zimmermann (Homburg, Germany)	Both 1:1000 for immunoblotting
Melan-A	Mouse mAb; Melan-A, Dako #7196	1:200 for immunohistochemistry
MCU	Rabbit mAb; MCU (D2Z3B); Cell Signal. Tech #14997	1:500 for immunoblotting
NCXL	Rabbit pAb; Anti-SLC24A6 antibody;	1:500 for immunoblotting

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