

2. Materials

2.1. Chemicals

Standard chemicals were ordered from Amersham (Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany), Biochrom (Berlin, Germany), Calbiotech (Bad Soden, Germany), Invitrogen (Karlsruhe, Germany), Merck (Darmstadt, Germany), Roche Biochemicals (Basel, Switzerland), Roth (Karlsruhe, Germany), Sigma-Aldrich (Sigma-Aldrich Chemie, Munich, Germany) in the quality pro Analysis (p.a.). For special chemicals and solutions, the manufacturer's name is indicated in the individual methods section. Buffers were prepared with millipore water.

2.2. Labware

All reusable labware was autoclaved before use. Plasticware was ordered from BD Biosciences (Heidelberg, Germany) or Eppendorf (Hamburg, Germany). Special labware used for this study was indicated in the corresponding section of methods.

2.3. Kits

PCR Cloning System with Gateway™ Technology (with pDONR.201™ and with pDONR221™) (Invitrogen)

Mammalian Expression System with Gateway™ Technology (Invitrogen)

QIAGEN Plasmid Mini kit (Qiagen)

QIAquick PCR purification kit (Qiagen)

EndoFree Plasmid Maxi Kit (Qiagen)

Effectene Transfection Reagent (Qiagen)

HiPerFect Transfection Reagent (Qiagen)

RNAiFect Transfection Reagent (Qiagen)

In Situ Cell Death Detection Kit, Fluorescein (TUNEL technology) (Roche)

Apoptosis sampler kit, #9915 (Rabbit antibodies against cleaved caspase-3, caspase-7, caspase-9 and PARP) (Cell Signaling Technology)

Annexin V-FITC Fluorescence Microscopy Kit (BD Biosciences Pharmingen)

CaspGLOW™ Fluorescein Active Caspase-3 Staining Kit (FITC-DEVD-FMK) (BioVision, California, USA)

CaspGLOW™ Fluorescein Caspase Staining Kit (FITC-VAD-FMK) (BioVision, California, USA)

2.4. Clones and vectors

The cDNA clones containing the Open Reading Frame (ORF) sequences of 89 human chromosome 21 genes were obtained from Human MTC Panels I+II and QUICK-Clone cDNA (Clontech, Heidelberg, Germany) or from public IMAGE or MGC cDNA clones (RZPD, Berlin, Germany).

pDONR™201 vector (Invitrogen)

pDEST™26 vector (Invitrogen)

pORF-hBax- α (InvivoGen, San Diego, USA)

pcDNA4/HisMax©-eGFP (a kind gift from Dominique Vanhecke in the same department)

pHcRed1-N1 vector (BD Biosciences Clontech, Inc.)

pDEST474 vector (a kind gift from Dominic Esposito, NCI-Frederick Vector Engineering Group, USA)

pENTRY clones for the KDELR1, PMP22, PMP26, LAPC1, lamin-A and TGN38 control proteins (a kind gift from Stefan Wiemann, German Cancer Research Center)

2.5. Oligonucleotides

2.5.1. DNA primers

Oligonucleotides used for the amplification of 89 Chr21 ORFs and for the construction of *attB*-site containing clones were ordered from Invitek (Berlin-Buch, Germany) (see Table A.1 and Table A.2 in Appendix).

2.5.2. siRNA oligonucleotides

The chemical synthesized siRNA oligonucleotides targeting 18 human genes and two control siRNAs were ordered from Qiagen Inc.

Table 2.1 Chemical synthesized siRNAs used in this study

Target Gene Symbol	Target Protein name	Target transcript	Target sequence/region	siRNA product Name
Negative control siRNA	none	none	AATTCTCCGAACGTGTCACGT	Control (non-sil.) siRNA, Rhodamine
Negative control siRNA	none	none	AATTCTCCGAACGTGTCACGT	Control (non-sil.) siRNA, AlexaFluor488
<i>AKT1</i>	RAC-alpha serine/threonine-protein kinase	NM_005163	1528-1578	<i>Hs_AKT1</i> validated siRNA
<i>BCL-2</i>	B-cell lymphoma protein 2	NM_000633	80-130	<i>Hs_BCL2(4)</i> validated siRNA
<i>PTEN</i>	Phosphatase and tensin homolog; mutated in multiple advanced cancers	NM_000314	1167-1217	<i>Hs_PTEN(3)</i> validated siRNA
<i>VIM</i>	Vimentin	NM_003380	1480-1540	<i>Hs_VIM(1)</i> validated siRNA
<i>AATF</i>	Apoptosis antagonizing transcription factor	NM_012138	CGGGAAGTGAGGAGATTTCTA	<i>AATF-2</i> siRNA
<i>ALS2CR2 (ILPIP)</i>	ILP-interacting protein ILPIPA (ILPIPA)	AY093697	TAGGATATATCTGCATCTTGA	<i>ILPIP-1</i> siRNA
			CAGGATTTACATGGGTATAAT	<i>ILPIP-2</i> siRNA
<i>BIRC2</i>	Baculoviral IAP repeat-containing protein 2; apoptosis inhibitor 1	NM_001166	AGGGATATAGTTTGAATTCTA	<i>BIRC2-1</i> siRNA
			AGGCATAATTTAGGTATTCTA	<i>BIRC2-2</i> siRNA
<i>BIRC4 (XIAP)</i>	Baculoviral IAP repeat-containing protein 4	NM_001167	ATGGAGCTTTCTGTATATAAAA	<i>BIRC4-1</i> siRNA
			AGGGTATAAACTAGAAAGTTTA	<i>BIRC4-2</i> siRNA
<i>BIRC5 (Survivin)</i>	Baculoviral IAP repeat-containing protein 5; survivin; apoptosis inhibitor 4	NM_001168	CTAGCGTAAGATGATGGATTT	<i>Survivin-1</i> siRNA
			CTCGGCTGTTCTGAGAAATA	<i>Survivin-2</i> siRNA
<i>BIRC7 (livin)</i>	Livin inhibitor of apoptosis	NM_022161	ATGCTTCTGAATAGAAATAAAA	<i>Livin-1</i> siRNA
			CAGGAGAGAGGTCCAGTCTGA	<i>Livin-2</i> siRNA
<i>CCNB1</i>	Cyclin B1	NM_031966	AAGAAATGTACCCTCCAGAAA	<i>siCyclinB1</i>
<i>DAXX</i>	Death-associated protein 6	NM_001350	GGAGTTGGATCTCTCAGAATT	<i>siDaxx</i>
<i>HNRPA1</i>	Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1)	NM_002136	AATGGGGAACGCTCACGGACT	<i>A1-1</i> siRNA
			AAATCATGACTGACCGAGGCA	<i>A1-5</i> siRNA
<i>HNRPA2</i>	Heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2)	NM_002137	AAGCTTTGAAAACACAGAAGA	<i>A2-1</i> siRNA
			AACCTTTGGTGGTAGCAGGAAC	<i>A2-5</i> siRNA
<i>HNRPA3</i>	Heterogeneous nuclear ribonucleoprotein A3 (hnRNP A3)	NM_194247	AGGCCCTTTCTAAACAAGAAA	<i>A3-1</i> siRNA
			GAGGAGGTGATGGTGGATATA	<i>A3-2</i> siRNA
<i>hSGTA</i>	Small glutamine-rich tetratricopeptide-containing, alpha, protein	NM_003021	AACTTTGAAGCTGCCGTGCAT	<i>hSGTA-1</i> siRNA
			AAGCACGTGGAGCCGTGGCT	<i>hSGTA-2</i> siRNA
<i>Lamin A/C</i>	Lamin A/C	NM_005572	AACTGGACTTCCAGAAGAACA	<i>Lamin A/C</i> siRNA
<i>OPA-1</i>	Optic atrophy 1	AB011139	AAGTTATCAGTCTGAGCCAGG	<i>OPA-1</i> siRNA

2.6. Cells

2.6.1. Bacterial (*E. coli*) stains

DH5 α TM (Invitrogen) and DH10B (Gibco life technologies, Invitrogen) competent cells were used for transformations of “entry” (pENTRY) and expression (pEXP) clones.

DB3.1TM Competent Cells was used for the transformation and propagation of GatewayTM Vectors.

2.6.2. Human cell lines

Human HEK293T/17 was ordered from ATCC[®] (Number: CRL-11268).

HeLa cell (ATCC[®] Number: CCL-2TM) (a kind gift from Sebastian Haesler, Max-Planck-Institute for molecular genetics, Department Human Molecular Genetics)

2.7. Antibodies and counterstains

Table 2.2 Primary antibodies and counterstains used in this study

Antibodies and Counterstains	Source	Supplier	Cat. No.
Annexin V15-Biotin	-	Caltag laboratories, Burlingame, CA	-
Annexin V-FITC	-	BD Biosciences Pharmingen	8074KC
Anti-Adaptin- γ (clone 88)	mouse IgG1	BD Biosciences Pharmingen	610385
Anti-Bax antibody (clone 3)	mouse IgG1	BD Biosciences Pharmingen	610983
Anti-calreticulin	rabbit polyclonal IgG	Abcam, Cambridge, UK	ab4
Anti-catalase	rabbit polyclonal IgG	Abcam, Cambridge, UK	ab1877
Anti-cleaved caspase-3 (Asp175)	rabbit polyclonal IgG	Cell Signaling	9661
Anti-cleaved caspase-7 (Asp198)	rabbit polyclonal IgG	Cell Signaling	9491
Anti-cleaved caspase-9 (Asp330)	rabbit polyclonal IgG	Cell Signaling	9501
Anti-cleaved PARP (Asp214)	rabbit polyclonal IgG	Cell signaling	9541
Anti-hnRNP A3	rabbit polyclonal IgG	gift from Karl Skriner (Charite, Germany)	-
Anti-human Lamin A+C (clone JoL2)	mouse Ig	CHEMICON international	MAB3211
Anti-LAMP2	mouse monoclonal IgG1	Developmental Studies Hybridoma Bank, Iowa City, IA, USA	H4B4
Anti-Myc Tag	rabbit polyclonal IgG	Upstate	06-549
Anti-PDI (clone 1D3)	mouse IgG1	Stressgen, San Diego, CA	SPA-891
Anti-prohibitin	rabbit polyclonal IgG	Abcam, Cambridge, UK	ab2996
Anti-tubulin (clone DM 1A)	mouse IgG1	Sigma, Missouri, USA	T 9026
Anti-Vimentin (clone J144)	mouse IgM	ABR Affinity BioReagents, Golden, CO	MA3-745
DAPI	-	Sigma	D-8417

Table 2.2 Primary antibodies and counterstains used in this study (continued)

Antibodies and Counterstains	Source	Supplier	Cat. No.
FITC-DEVD-FMK (FLICA staining active Caspase-3)	-	Biovision, CA, USA	K183-25
FITC-VAD-FMK (FLICA staining pan-Caspases)	-	Biovision, CA, USA	K180-25
Lectin GS-II from <i>Griffonia simplicifolia</i> , AlexaFluor488 conjugate	-	Molecular Probes Invitrogen	L-21415
Rhodamine Phalloidin	-	Molecular Probes Invitrogen	R-415
FITC-TUNEL reaction (In Situ Cell Death Detection Kit)	-	Roche Diagnostics GmbH, Penzberg, Germany	11 684 795 910
Anti-His tag antibody	mouse monoclonal IgG1	DPC Biermann	SM1693P
Anti-His tag antibody	goat polyclonal IgG	Abcam, Cambridge, UK	ab9136
Anti-His(C-term) antibody	mouse monoclonal IgG2b	Invitrogen	R930-25
Anti-HisG antibody	mouse monoclonal IgG2a	Invitrogen	R940-25
Penta•His AlexaFluor 488 antibody	mouse monoclonal IgG1	Qiagen	35310
Penta•His AlexaFluor 555 antibody	mouse monoclonal IgG1	Qiagen	35350
Penta•His antibody	mouse monoclonal IgG1	Qiagen	kit 34698
RGS•His antibody	mouse monoclonal IgG1	Qiagen	kit 34698
Tetra•His antibody	mouse monoclonal IgG1	Qiagen	kit 34698

PARP: poly(ADP-ribose) polymerase; hnRNP A3: heterogeneous nuclear ribonucleoprotein A3; LAMP2: Lysosome-associated membrane glycoprotein 2 [Precursor] ; PDI: protein disulfide isomerase; FLICA: fluorochrome-labeled inhibitors of caspases; TUNEL: TdT-mediated dUTP nick end labeling

Table 2.3 Secondary antibodies used for protein colocalization and cell death detection

Antibodies and Counterstains	Source	Supplier	Cat. No.
AlexaFluor488-anti-Fluorescein/Oregon Green	rabbit polyclonal IgG	Molecular Probes Invitrogen	A-11096
AlexaFluor488-anti-Mouse IgG (H+L)	goat F(ab') ₂	Molecular Probes Invitrogen	A-11017
AlexaFluor568-anti-Mouse IgG (H+L)	goat F(ab') ₂	Molecular Probes Invitrogen	A-11019
AlexaFluor488-anti-Rabbit IgG (H+L)	donkey	Molecular Probes Invitrogen	A-21206
AlexaFluor555-anti-Rabbit IgG (H+L)	goat F(ab') ₂	Molecular Probes Invitrogen	A-21430
Cy ³ -conjugated anti-rabbit IgG (H+L)	mouse	Jackson ImmunoResearch	211-165-109
AlexaFluor594-Streptavidin	-	Molecular Probes Invitrogen	S32356
AlexaFluor488-Streptavidin	-	Molecular Probes Invitrogen	S32354

2.8. Software, Databases, and Bioinformatic tools

Table 2.4 Databases used in this study

Database name	Database type	Weblinks
EntrezGene	Gene database	http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Unigene	Gene-oriented sequence clusters	http://www.ncbi.nlm.nih.gov/UniGene
GenBank	Sequence retrieval and analysis	http://www.ncbi.nlm.nih.gov/Genbank/
dbSNP	Signal nucleotide polymorphism	http://www.ncbi.nlm.nih.gov/SNP
Ensembl	Sequence retrieval and analysis	http://www.ensembl.org
Chromosome 21 gene catalogue	Sequence retrieval and analysis	http://chr21.molgen.mpg.de/chr21_catalog9.html
EntrezProtein	Protein database	http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein
Swiss-Prot/TrEMBL	Protein database	http://www.expasy.org/sprot/
Pfam	Protein families	http://www.sanger.ac.uk/Pfam
Smart	Protein families	http://smart.embl-heidelberg.de
Prosite	Protein families and domains	http://www.expasy.org/prosite
OMIM	Genetically linked diseases	http://www.ncbi.nlm.nih.gov/omim/

Table 2.5 Bioinformatic tools and software used in this study

Name	Application in this study	Weblinks and Companies
PSORTII	Protein sorting and localization sites	http://psort.ims.u-tokyo.ac.jp/form2.html
PromptComp v. 5	Protein localization prediction	http://www.softberry.com
PENCE Proteome Analyst v1.0	Protein localization prediction	http://www.cs.ualberta.ca/%7Ebioinfo/PA/Su b/index.html
SubLoc v1.0	Protein localization prediction	http://www.bioinfo.tsinghua.edu.cn/SubLoc/
PRIDE	Primer design	http://pride.molgen.mpg.de/pride.html
Axiovision 4.0 software	Fluorescent microscopy image analyses	Zeiss, Jena, Germany
LSM510 software	Confocal image analyses	Zeiss, Jena, Germany
GenePix 6.0	Image analyses and signal quantification	Axon Laboratory