# 9 Index

# 9.1 Figure index

Figure 2-1: Regulation of the eukaryotic cell cycle	9
Figure 2-2: Acquired capabilities of cancer	. 11
Figure 2-3: Growth factor signaling	. 12
Figure 2-4: Consensus sequence of prohibitin proteins and their putative domains	. 13
Figure 2-5: Prohibitin/m-AAA complex in the inner mitochondrial membrane	. 17
Figure 2-6: Processing of OPA1 is m-AAA dependent	. 19
Figure 2-7: OPA1 requires PARL and m-AAA	. 20
Figure 2-8: Growth factor signaling in migration	. 21
Figure 2-9: Protrusion formation upon activation of growth factor receptors	. 22
Figure 2-10: Cell-cell contacts and cell matrix contacts	. 23
Figure 2-11: Signaling in cell-cell and cell-matrix contacts	. 24
Figure 4-1: Prohibitin shRNAs and control shRNAs were cloned into the pLL3.7 vector system	. 26
Figure 4-2: Expression of the shRNAs shPHB, shPHBy and shPHBz	. 27
Figure 4-3: Prohibitin knockdown leads to reduced GFP expression in different cancer cells	. 29
Figure 4-4: Reduced BrdU uptake in HeLa cells expressing prohibitin shRNAs	. 30
Figure 4-5: DNA staining with propidium iodide did not reveal a block in cell cycle progression	. 31
Figure 4-6: Prohibitin interacts with C-Raf via its c-terminal Raf binding domain	. 32
Figure 4-7: The block of proliferation is independent of growth factor signaling	. 33
Figure 4-8: Loss of prohibitin stops colony growth in soft agar	. 34
Figure 4-9: The pLVPT one vector, doxycycline inducible lentiviral vector system	. 35
Figure 4-10: Transduced HeLa cells show a doxycycline inducible knockdown of prohibitin	. 36
Figure 4-11: Double knockdown of prohibitin proteins through loss of protein stability	. 37
Figure 4-12: Decrease of CFSE intensity is slowed down upon prohibitin knockdown	. 38
Figure 4-13: ATP levels are not changed in prohibitin knockdown cells	. 39
Figure 4-14: A prohibitin knockdown does not lead to a destabilization of proteins from the elec-	tron
chain complexes but rather to change in the expression pattern of the fusion protein OPA1	. 40
Figure 4-15: Expression of shPHBz resulted in an increase in mitochondria fragmentation	. 41
Figure 4-16: Mitochondrial fragmentation in shPHBz cells correlates with changed OPA1 pattern	1.42
Figure 4-17: TMRE staining is not reduced in prohibitin knockdown cells	. 43
Figure 4-18: OPA 1 expression pattern was completely changed in CCCP treated HeLa cells	. 44
Figure 4-19: Comparison of OPA1 fragmentation in shPHBz cells and CCCP treated cells	. 44
Figure 4-20: A prohibitin knockdown in HeLa cells leads to aberrant cell spreading	. 45
Figure 4-21: Aberrant spreading correlates with an overexpression of EGFR on the cell surface.	. 46
Figure 4-22: Prohibitin knockdown cells showed reduced adhesion to fibronectin and collagen	. 47
Figure 4-23: Under suspension conditions, prohibitin k.d. cells did not grow and form colonies	. 48
Figure 5-1: Processing of OPA1 by m-AAA, Parl or a 3 <sup>rd</sup> unknown protease	. 57

## 9.2 Abbreviations

miscellaneous abbreviations				
Å	Ångstrom			
aa	Amino acids			
APS	ammonium persulfate			
BrdU	5-bromo-2'-deoxyuridine			
BSA	bovine serum albumin			
С	cytosine			
CCCP	Carbonyl cyanide 3-chlorophenylhydrazone			
CFSE	Carboxy fluorescein diacetate succinimidyl ester			
Ci	Curie			
dGDP	desoxy Guanosine Di-Phosphate			
dGTP	desoxy Guanosine Tri-Phosphate			
DMEM	Dulbecco modified Eagle Serum			
DMSO	dimethylsulfoxide			
DTT	dithioerythreitol			
ECM	extra cellular matrix			
E.coli	Escherichia coli			
EDTA	ethylene diamine tetra acetic acid			
e.g.	for example (latin: 'exempli gratia')			
EGF	epidermal growth factor			
EGTA	ethylene glycol tetra acetic acid			
ELISA	enzyme linked immunosorbent assay			
FACS	fluorescence activated cell sorter			
FCS	Fetal calf serum			
$G_1/G_2$	gap phase 1/ gap phase 2			
HBS	Hanks' balanced salt solution			
HEPES	(N-/2-hydroxyethyl) piperazin-N'-2-ethansulfon acid			
IB / IF	Immunoblot / Immunofluorescence			
IM	mitochondrial inner membrane			
IMS	mitochondrial inner membrane space			
IPTG	isopropylthiogalactosid			
IRES	internal ribosomal entry side			
k.d.	knockdown			
KRAB	Krüppel-associated box			
mAb	Monoclonal antibody			
MeOH	methanol			
MMP	mitochondrial membrane potential			

#### miscellaneous abbreviations

MOI multiplicity of infection

MTOC microtubule organizing center

N Nucleotide (A, C, G or T)

OD optic density

OM mitochondrial outer membrane

o/n over night

pAb Polyclonal antibody

p.i.

p.i.

PFA para fromaldehyde
PI propidium iodide

PMA phorbol 12-myristate 13-acetate
PVDF polyvinylidenfluoridmembrane

qRT-PCR quantitative reverse transcriptase polymerase chain reaction

R restriction point

RPMI Roswell park memorial institute

RT room temperature

35S isotope 35 of sulfur
S / S-phase synthesis phase

SDS sodium dodecyl sulfate
siRNA small interfering RNA
shRNA small hairpin RNA

SNP single nucleotide polymorphism

SSC side scatter
T thymine

TAE Tris acetic acid EDTA

TBS Tris-buffered saline w/ EGTA

TBS-T Tris-buffered saline with 0.05% Tween 20

TCA Trichloroacetic acid

TEMED N, N, N', N',-tetramethylendiamin

TMRE Tetramethylrhodamine ethyl ester

TNF tumor necrosis factor
TU transduction units
UTR untranslated region

w/ with

#### abbreviations of genes and proteins

Bap37 B-cell associated protein 37kDa-prohibitin

Ccp1p yeast – cyctochrome *c* peroxidase protein
CDKC cyclin dependent kinase (CDK) complex

CIP CDK inhibitor protein

Cobp yeast - cytochrome b

Cox1p yeast - cytochrome c oxidase

Drp1 dynamin-related GTPase
E2F gene regulatory protein

EGFR epidermal growth factor receptor

ErbB2 v-erb-b2 erythroblastic leukemia viral oncogene homolog 2

Erk extracellular signal regulated protein kinase

GAPDH glycerinaldehyd-3-phosphat-dehydrogenase

GEF guanine exchange factor

Grb2 growth factor receptor-bound protein 2

GST glutathione-S-transferase
GTPase guanosine tri-phosphatase

Her2 human epidermal growth receptor 2

Hsp60 heat shock protein 60 kDa

i-AAA inner membrane space ATP associated with diverse cellular acivity (AAA) protease

IgM immunoglobulin M

m-AAA matrix ATP associated with diverse cellular acivity (AAA) protease

Mfn mitofusin

MEK MAPK/Erk kinase

Mgm1p yeast ortholog to OPA1

OPA1 optic atrophy type 1

PARL presenilin-associated rhomboid-like

PHB prohibitin

Phb1p / Phb2p yeast –prohibitin 1 protein / yeast –prohibitin 2 protein

Raf ras-activated factor

Ras rat sarcoma

Rbd1p yeast ortholog to PARL

REA repressor of estrogen receptor acitivity –prohibitin 2

RTK receptor tyrosin kinase

Tim23 translocase of the inner mitochondrial membrane (23kDa)

Yta10p yeast homolog to paraplegin protein
Yta12p yeast homolog to AFG3L2 protein

## 9.3 List of constructs

	construct name	vector name	domain/mutation
pCla001		pLL3.7	shRNA
pCla002	pLL3.7-shPHB	pLL3.7	shRNA
pCla018	PHB1-272-GST	pGex4T3	aa 1-272
pCla019	PHB1-243-GST	pGex4T3	aa 1-243
pCla028	PHB1-272 His	pET28c	aa 1-272
pCla030	PHB1-243 His	pET28c	aa 1-243
pCla036	PHB-eGFP	pEGFP-N1	aa 1-272
pCla043	pLL3.7-shPHBx	pLL3.7	shRNA
pCla044	pLL3.7-shPHBy	pLL3.7	shRNA
pCla045	pLL3.7-shPHBz	pLL3.7	shRNA
pCla046	pLL3.7-shPHB2-0	pLL3.7	shRNA
pCla047	pLL3.7-shPHB2-1	pLL3.7	shRNA
pGB001	pLVPT-shLuci	pLVPT-tTRKRAB	shRNA
pGB002	pLVPT-shPHB2-0	pLVPT-tTRKRAB	shRNA
pGB003	pLVPT-shPHB2-1	pLVPT-tTRKRAB	shRNA
pGB004	pLVPT-shPHBy	pLVPT-tTRKRAB	shRNA
pGB005	pLVPT-shPHBz	pLVPT-tTRKRAB	shRNA
pGB006	pLVPT-shPHB	pLVPT-tTRKRAB	shRNA
	C-Raf-HA	pcDNA3	
	C-Raf-GST	pGex3T3	
	HeLa-PHB 3'UTR	pGL3-control	PHB 3'UTR
	HT1080-PHB 3'UTR	pGL3-control	PHB 3'UTR
	IF6-PHB 3'UTR	pGL3-control	PHB 3'UTR
	Capanl-PHB 3'UTR	pGL3-control	PHB 3'UTR
	IMR-E1A-PHB 3'UTR	pGL3-control	PHB 3'UTR

### **Publications**

Rajalingam, K., Wunder, C., Brinkmann, V., Churin, Y., Hekman, M., Sievers, C., Rapp, U.R. and Rudel, T. Prohibitin is required for Ras induced Raf-MEK-ERK activation and epithelial cell migration. Nat Cell Biol. 2005 Aug; 7(8): 837-43

Rubinson, D.A., Dillon, C.P., Kwiatkowski, A.V., Sievers, C., Kopinja, J., McManus, M.T., Gertler, F.B., Scott, M., and Van Parijs, L. Functional and stable gene silencing in transgenic mice and primary mammalian cells by lentivirus-induced RNA interference. Nat Genet. 2003 Mar;33(3):401-6

#### **Presentation**

Using RNAi to dissect apoptosis pathways. Sievers, C., Dillon, C.P., Rubinson, D.A. and Van Parijs, L. Presented at New England Immunology Conference. Nov. 16th, 2002.

### 9.5 Acknowledgments

My thanks go to Priv. Doz. Dr. Thomas Rudel for supervising this thesis and providing such an interesting project under excellent working conditions.

Thanks to Prof. Petra Knaus for supervising this thesis as a second reviewer and providing a great seminar with room for fruitful discussions.

Most of all I want to thank my labmates, especially Nouhad Benlasfer, Kathleen Gottschalk and Manu Sharma, not only for excellent technical support, discussions and sharing of good ideas, but also for the spare time coffee and after-work beer with movies.

Thanks to Gwendolyn Billig, my benchmate, who had the honor of being the first diploma student I supervised.

Many thanks go to Dr. Vera Kozjak-Pavlovic, Dr. Nikolaus Machuy and Dr. Yoshan Moodley for scientific advice and help on and offside the bench through the past years or simply for adding the finishing touches to the manuscript.

Many people, who were not directly involved in helping me finishing this PhD thesis, because most of them don't even have a clue about science, probably took the main part in letting me survive the last years: my friends. Thank you