5. Materials

5.1 Reagents

Salts and buffer reagents were purchased in analytical quality from Life Technologies (Karlsruhe, D), Merck (Darmstadt, D), Roth (Karlsruhe, D) or Sigma (Munich, D). Solutions and solvents were purchased from J.T.Baker (Deventer, NL), Merck (Darmstadt, D), Roth (Karlsruhe, D) or Sigma (Munich, D). Gases were purchased from Linde (Berlin, D). Enzymes for molecular biology were obtained from Life Technologies (Karlsruhe, D).

Special agents were purchased as listed below:

N-acetylsphinganine (C_2-dihydroceramide)	Biomol (Hamburg, D)
N-acetylsphingosine (C_2-ceramide)	Alexis (Grunberg, D)
agarose	Roth (Karlsruhe, D)
DNA-marker (ΦX174 / HaeIII-fragments)	Life Technologies (Karlsruhe, D)
ethidium bromide	Biomol (Hamburg, D)
leupeptin	Sigma (Munich, D)
1-palmitoyl-2-[1-^{14}C]-palmitoyl-L-3-phosphatidyl-choline in toluene:ethanol 1:1 (v/v)	Amersham Pharmacia (Braunschweig, D)
pepstatin	Sigma (Munich, D)
phosphatidylethanolamine	Sigma (Munich, D)
phosphatidylinositol-4,5-bisphosphate	Sigma (Munich, D)
SulfoLink kit	Pierce (Weiskirchen, D)
SuperSignal chemiluminescence reagent	Pierce (Weiskirchen, D)

consumable goods were purchased as listed below:

Centricon centrifuge tubes	Amicon (Danvers, MA, USA)
nitrocellulose membrane	Schleicher & Schüll (Dassel, D)
RNeasy kit	Qiagen (Hilden, D)
QIAShredder	Qiagen (Hilden, D)
scientific imaging films X-OMAT AR	Kodak (Berlin, D)
silica gel 60 HPTLC plates	Merck (Darmstadt, D)

5.2 Cell culture materials

Sterile disposable materials were from

cell culture flasks, centrifuge tubes, chamber slides	Nunc (Wiesbaden, D)
Dulbecco´s modified Eagle's medium (DMEM)	Life Technologies (Karlsruhe, D)
foetal calf serum (FCS)	Seromed-Biochrom (Berlin, D)
human serum	Biotest (Dreieich, D)
Penicillin/Streptomycin	Seromed-Biochrom (Berlin, D)
phosphate buffered saline (PBS)	Seromed-Biochrom (Berlin, D)
pipettes	Costar (Cambridge, MA, USA)
Roswell Park Memorial Institute 1640 (RPMI 1640) medium	Seromed-Biochrom (Berlin, D)
6-well-plates, 24-well-plates	Corning (New York, NY, USA)
sterile filters	B.Braun (Melsungen, D)
trypsin	Seromed-Biochrom (Berlin, D)

5.3 Cell lines

Two melanoma cell populations M186 and M221 were obtained from patients with histologically confirmed metastatic melanoma by surgical intervention. The human melanoma cell line A375 (GIARD *et al.*, 1973) originated from primary tumours and Mel2A (BRUGGEN *et al.*, 1981) originated from metastases. The immortalised human keratinocyte cell line HaCaT (BOUKAMP *et al.*, 1988) was kindly provided by Prof. N. E. Fusening, Deutsches Krebsforschungszentrum (Heidelberg, D). The human leukaemia cell line HL60 was purchased from American type culture collection (Rockwell, ML, USA).

5.4 Antibodies

anti ADP-ribosylation factor antibody (H-50, rabbit)	Santa Cruz Biotechnology (Heidelberg, D)
alkaline phosphatase anti alkaline phosphatase complexes (mouse)	Dako (Hamburg, D)
anti caspase-3 polyclonal antibody (rabbit)	Pharmingen (Hamburg, D)
anti CD95 agonistic monoclonal antibody (CH-11, mouse)	Chemicon (Hofheim ,D)
anti goat antibody coupled with horseradish peroxidase (rabbit)	Dako (Hamburg, D)
anti mouse antibody coupled with horseradish peroxidase (goat)	Dako (Hamburg, D)
anti mouse antibody (rabbit)	Dako (Hamburg, D)
anti phospholipase D1 (αNChPLD1, rabbit)	MÜLLER-WIEPRECHT *et al.* (1998)
anti phosphotyrosine (PY-20, mouse)	Calbiochem (Bad Soden, D)
anti protein kinase Cα antibody (H-7, mouse)	Santa Cruz Biotechnology (Heidelberg, D)
anti rabbit antibody coupled with horseradish peroxidase (goat)	Dako (Hamburg, D)
anti rabbit antibody (mouse)	Dako (Hamburg, D)
anti rhoA antibody (26C4, mouse)	Santa Cruz Biotechnology (Heidelberg, D)

5.5 Equipment

balances	474	Kern (Albstadt, D)
	1419	Sartorius (Gottingen, D)
centrifuges	Biofuge fresco	Heraeus (Osterode, D)
	Biofuge pico	Heraeus (Osterode, D)
	Labofuge 400E	Heraeus (Osterode, D)
	T-2050	Centrikon (Rotkreuz, CH)
	Ultracentrifuge J2-21	Beckman Instruments (Glenrothes, GB)
	Varifuge RF	Heraeus (Osterode, D)
imaging densitometer	Model GS-700	Bio-Rad (Munich, D)
CO_2-incubator	BB16	Heraeus (Osterode, D)
drying oven	T6	Heraeus (Osterode, D)
electrophoresis chambers	GNA-200	Pharmacia (Freiburg, D)
	Mini Protean II	Bio-Rad (Munich, D)
	Mini Sub Cell	Bio-Rad (Munich, D)
ELISA photometer	Model 550	Bio-Rad (Munich, D)
gel-documentation unit		Polaroid (Offenbach, D)
incubation shaker	Aerotron AI 18	Infors (Bottmingen, CH)
incubator	B 15	Heraeus (Osterode, D)
laminar flow bench	BSB 4 A	Gelaire Flow Laboratories (Opera, I)
magnetic stirrer	MR 2000	Heidolph (Kelheim, D)
	Monotherm	Faust (Cologne, D)
microscope	Diavert	Leitz (Wetzlar, D)
	BX60F5	Olympus (Tokyo, J)
microwave oven	Privileg 8017	Quelle (Furth, D)
mixer	Thermomixer comfort	Eppendorf (Hamburg, D)
	Vortex IKA VF2	Jahnke und Kunkel (Staufen i. Br., D)
orbital shaker	WT 17	Biometra (Gottingen, D)
phosphorimager	BAS 1500	Fuji (Dusseldorf ,D)

phosphorimager plates	BAS IIIs	Fuji (Dusseldorf ,D)
power supplies	ECPS 3000/150	Pharmacia (Freiburg, D)
	Model 1000/500	Bio-Rad (Munich, D)
pH-meter	pH 526 incl. SenTix 97 T	WTW (Weilheim i. OB, D)
thin layer chromatography applicator	Linomat IV	Camag (Muttenz, CH)
thin layer chromatography separating chambers	. . . . .	Desaga (Heidelberg, D)
thermocycler	GeneAmp PCR System 2400	Perkin Elmer (Weiterstadt, D)
ultrasonic bath	Sonorex RK 52	Bandelin (Berlin, D)
UV-Vis photometer	Ultrospec 1000	Pharmacia (Freiburg, D)
water bath	1002	GFL (Burgwedel, D)
Western-blot transfer apparatus	Mini Trans-Blot	Bio-Rad (Munich, D)

5.6 Primer

Following oligonucleotides were synthesised by TIB Molbiol (Berlin, D). Sequences, amplicon length, annealing temperature and number of cycles are listed in the table.

gene	primer sequence	amplicon length	annealing temperature	number of cycles
ribosomal protein S9 5'	5'-GAG ACA ATC CAG CAG CCC AGG AGG GAC A-3'	431 bp	60 °C	24
ribosomal protein S9 3'	5'-GAT GAG AAG GAC CCA CGG CGT CTG TTC G-3'			
ß-actin 5'	5'-TCC TCC CTG GAG AAG AGC TA-3'	388 bp	55 °C	20
ß-actin 3'	5'-TCA TAC TCC TGC TTG CTG AT-3'			
c-jun 5'	5'-TGC AAA GAT GGA AAC GAC CTT C-3'	315 bp	55 °C	32
c-jun 3'	5'-TGC TCA TCT GTC ACG TTC TTG G-3'			
c-fos 5'	5'-TCG GGG TAG GTG AAG ACG AA-3'	869 bp	69 °C	28 (2 step)
c-fos 3'	5'-GCC AAC TTC ATT CCC ACG GT-3'			
fosB 5'	5'-AAA TGC CCG GTT CCT TCG TG-3'	453 bp	60 °C	33
fosB 3'	5'-CCG ACT CCA GCT CTG CTT TT-3'			
involucrin 5'	5'-CAT TCA ACC AGC CCT GCC CA-3'	319 bp	60 °C	33
involucrin 3'	5'-TAG AGG CTC CGC TCA CCT GA-3'			
loricrin 5'	5'-AGC ATA ATG AAG GCT TTC TG-3'	217 bp	54 °C	32
loricrin 3'	5'-ACA TCT TGT CTG AGA AGG AA-3'			
transglutaminase 1 5'	5'-AGT ATG AGT ACG ACG AGC TG-3'	440 bp	55 °C	26
transglutaminase 1 3'	5'-GGT CCC GTA GTA AAT TCT CC-3'			
tissue transglutaminase 5'	5'-ACA GTC TCA CCT TCA GTG TC-3'	539 bp	56 °C	28
tissue transglutaminase 3'	5'-CTG GTC ATC GTT GCA GTT GA-3'			
keratin V 5'	5'-TCA CTG TCA ACC AGA GTC TC-3'	750 bp	50 °C	33
keratin V 3'	5'-TCA TCC GGT TCA TCT CTG TG-3'			
phospholipase D1(abc) 5'	5'-AAA AGC ACA ACA AGG AGT GA-3'	1064 bp	53 °C	32
phospholipase D1(abc) 3'	5'-TGG ACT CTT CAT GGT ACT TT-3'			
phospholipase D1a 5'	5'-CAA ACT CTT TCA CCC GTC CA-3'	627 bp	55 °C	31
phospholipase D1b 5'	5'-ATT GAC AGC ACC TCC AAT AC-3'	587 bp	55 °C	26
phospholipase D1 3'	5'-TGG TTT TCC CTG TGA GCT TT-3'			
phospholipase D2 5'	5'-AGT CTG CGG AAG CAC TGC TT-3'	315 bp	60 °C	28
phospholipase D2 3'	5'-GGG CAG CAA AGA CTC ATC CT-3'			
rhoA 5'	5'-GAG GCT GGA CTC GGA TTC GT-3'	642 bp	62 °C	22
rhoA 3'	5'-CCG CAT AAG GGC TGT GCT TG-3'			
rhoB 5'	5'-AGC CCG AGG TGA GCA GTG AG-3'	763 bp	70 °C	35 (2 step)
rhoB 3'	5'-GGA CAC GGG TCT CCC CTT CT-3'			
rhoC 5'	5'-GAC TTC ATC TCA GCT CCA GA-3'	727 bp	56 °C	27
rhoC 3'	5'-GGG TTG TAG GGG GAT AAT TT-3'			