Appendix S1

MATERIALS AND METHODS

Cell culture. All experiments were performed according to the principles of the Declaration of Helsinki and approved by the ethics committee at Linköping University, Linköping, Sweden. Pure melanocyte cultures were established as described previously (30) from foreskins obtained from Caucasian donors (0–2 years of age). The melanocytes (MC) were cultured in medium 199 with 2% foetal bovine serum (FBS), supplemented with 50 U/ml penicillin, 50 µg/ml streptomycin, 50 µg/ml amphotericin B (Fungizone®), 10 ng/ml basic fibroblast growth factor, 10 µg/ml inositol (all from Invitrogen, Paisley, UK), 10 µg/ml insulin, 0.1 nM cholera toxin, 0.4 µg/ml hydrocortisone, 1 nM tri-iodothyronine, 10 µg/ml transferrin (all from Sigma Aldrich, St Louis, USA) and 10 ng/ml epidermal growth factor (31). Cells in passages 2–5 were used for experiments and no cells were cultured for more than 3 weeks in total. Untreated controls from the same individual were analysed in parallel.

FM55P, derived from a primary melanoma, and 2 metastasis cell lines (FM55M1, FM55M2) established from the same individual were used (a gift from Prof Meenhard Herlyn, Wistar Institute, Philadelphia, USA). The melanoma cells were cultured in RPMI 1640 with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml amphotericin B and 2 mM L-glutamine (all from Invitrogen). The melanoma cells were cultured in 2% FBS 24 h prior experiments. When indicated, inhibitors of cathepsin B (CA-074 Me; 1 µM), cathepsin L (inhibitor I; 10 µM), cathepsin K (inhibitor II; 10 µM; all from Calbiochem, San Diego, CA, USA), and cathepsin D (pepstatin A; 100 µM, Sigma Aldrich) were used.

Database analysis. Data were extracted from the E-GEOD-3189 experiment in the Gene Expression Atlas (www.ebi.ac.uk/gxa/), which provides Robust Multi-array Average-normalized expression values from published data-sets. This experiment included a total of 70 samples consisting of 45 MMs, 18 benign naevi and 7 normal samples (32). The mean levels of the probes corresponding to the cathepsin B (200838_at, 213274_s_at, 200839_s_at), cathepsin D (200766_at), cathepsin K (202450_s_at) and cathepsin L (202087_s_at) mRNAs were calculated. Employing the Human Protein Atlas, the immunohistochemical scores for cathepsins B, D and L in 8–12 different human melanomas were extracted. The validation score was based primarily on the conformance of the expression pattern to the available gene/protein characterization data in the scientific literature and to data from bioinformatic predictions (33). Cathepsin K data was extracted from 85 MMs (21).

UV irradiation. The UVA source was a Medisun 2000-L tube (Dr Gröbel UV-Elektronik GmbH, Ettlingen, Germany; 340–400 nm) with a UVA output of 80 mW/cm^2 measured by a psoralen plus ultraviolet A (PUVA) Combi Light dosimeter (Leuven, Belgium). The UVB source was a Philips TL20W/12 tube (Philips, Eindhoven, Netherlands) emitting in the spectral range 280–370 nm with a main output between 305 and 320 nm; a Schott WG 305 cut-off filter (Mainz, Germany) was used. UV exposure was performed in phosphate-buffered saline (PBS) using a dose of 10 J/cm^2 UVA and 500 mJ/cm^2 UVB.

Western blot analysis. For analysis of the extracellular protein levels, cell culture media was collected and concentrated using Amicon Ultra centrifugal filter devices (Millipore, Billerica, MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-HCl Ready gel (Bio-Rad Laboratories) and then transferred to a Hybond™-P polyvinylidene fluoride blotting membrane (Amersham Biosciences, Buckinghamshire, UK). The blots were saturated with 5% non-fat dried milk (Bio-Rad Laboratories) in PBS supplemented with 0.05% Tween 20. Primary antibodies targeting cathepsins B, L, D (Athens Research & Technology, Athens, Greece) and K (Abcam, Cambridge, UK) and lysosomal associated membrane protein-1 (LAMP-1) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were incubated for 2 h at room temperature, followed by HRP-conjugated secondary antibodies (Amersham Biosciences) overnight. The bands were visualized using the ECL-Plus Western blotting detection system (Amersham Biosciences), and the membranes were re-probed with anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Biogenesis, Poole, UK) as an internal control.

Cell invasion. Before experiments, the cells were cultured for 24 h in serum-free medium. The top chamber membranes (Cultrex BME cell invasion assay, R&D Systems) were coated with BME solution at 37°C overnight. Cells were then seeded into the top chambers of the devices. Medium supplemented with 10% FBS was placed in the bottom chamber. Following 24 h of incubation, cells that successfully invaded the BME and migrated through the membrane were stained with calcein AM solution, dissociated and collected in the lower chamber. Fluorescence was measured at λ_{ex} 485 nm/λ_{em} 520 nm.

Proliferation. Cells seeded at a low density were cultivated over a period of 8 days (MC) or 3 days (melanoma cells), fixed with 4% paraformaldehyde (PFA) for 20 min and stained with 0.4% crystal violet (Sigma Aldrich) for 20 min. After being washed and dried, crystal violet was dissolved in 1% SDS (sodium dodecyl sulphate). Absorbance was measured at 550 nm. Cathepsin inhibitors were added to the culture medium, as described above, every second day. The selected inhibitor concentrations were not toxic to the cells.

To assess proliferation in medium from irradiated cell cultures, we irradiated confluent cultures in phenol-free cell culture medium without serum and additives. Culture medium was irradiated in dishes without cells as a control. The irradiated medium was collected immediately and supplemented with serum and additives before it was added to cells. Every second day, the culture medium was changed using irradiated medium collected from a new set of confluent cultures.

Plasma membrane repair. Cell cultures were irradiated in PBS with or without supplementation with Ca^{2+} (0.9 mM $CaCl_2$). The Ca^{2+}-free PBS was supplemented with 10 mM EGTA. Five minutes after UV irradiation, 5 µg/ml propidium iodide (PI) (Sigma Aldrich) was added, and the cultures were incubated for additional 5 min before they were fixed in 4% PFA (20 min, 4°C) and mounted in ProLong Gold Antifade Reagent supplemented with DAPI (4',6-diamidino-2-phenylindole) (1.5 µg/ml; Molecular Probes). Specimens were analysed using a fluorescence microscope (Olympus, Hamburg, Germany).

Immunocytochemistry. Five minutes after UV exposure, the cells were incubated in PBS supplemented with 5% FBS for 5 min at 4°C to inhibit endocytosis. Then, the cells were incubated with an anti-human LAMP-1 antibody (directed toward the luminal part of the protein, 1:50, Santa Cruz Biotechnology) for 1 h at room temperature. The cells were fixed in 4% PFA for 20 min at 4°C and then incubated with a secondary anti-goat antibody conjugated to Alexa Fluor 488 (1:100, Molecular Probes) for 1 h at room temperature. The samples were mounted in ProLong Gold antifade reagent with DAPI and analysed using a Zeiss LSM confocal microscope. Incubation with the secondary antibody was performed as a negative control for non-specific binding.

Statistics. Statistical comparisons were performed with 1-way analysis of variance (ANOVA) in Fig. 4b and with Student's *t*-test and Bonferroni correction in Fig. 1a, 3d, 4d and 4e. *p*-values < 0.05 were considered significant.