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 μM 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 triiodothyronine, 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 Mc; 1 μM), cathepsin L (inhibitor I; 10 μM), cathepsin K (inhibitor II; 10 μM; all from Calbiochem, San Diego, CA, USA), and cathepsin D (pepsatin 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/ega/), 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 UV source was a Medisun 2000-L (1.5 μg/ml; Molecular Probes). Specimens were analysed using a Zeiss LSM confocal microscope. Incubation with the secondary antibody was performed as a negative control for localization. The Ca2+-free PBS was supplemented with 10 mM EGTA. Five minutes after UV exposure, the cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a 15% Tris-MA, USA). For intracellular protein measurements, cells were lysed in RIPA lysis buffer, separated on a