Supplementary Methods

DDB2 knockdown by short hairpin RNA

Lentiviral particles shRNA containing either 3 target-specific constructs that encode short hairpin RNA (shRNA) ensured by manufacturer for knockdown DDB2 (sc-37799-V) gene expression or nontargeting control sequences (sc-108080) were purchased from Santa Cruz Biotechnology, Inc. Breast tumor T47D cell line was infected with lentiviral shRNA as recommended by the manufacturer and after puromycin selection, stable selected cells were used for the experiments. Efficiency of DDB2 knockdown was determined in resistant cells by RT-PCR and western blot analyses.

Antibodies

Rabbit polyclonal anti-DDB2, anti-β-actin, anti-p50NF-κB, anti-p65NF-κB (Santa Cruz Biotechnology, Inc.), and anti-IκBα (Sigma-Aldrich). Secondary horseradish peroxidase- and Alexa 594-conjugated antibodies were obtained from Sigma-Aldrich and Invitrogen, respectively.

Wound healing assay

Migration was studied by wound healing assay. An artificial wound was done using a 1000 µL pipette tip on confluent cell monolayers in 6-well culture plates in serum-containing medium. Cell migration into the wound area was observed and images were taken at 0 and 36 hours with phase-contrast microscope (Motic) equipped with a camera (Moticam 2300). Three independent experiments were done. Wound healing assay was done with cells treated with 50 µg/mL SN50 peptide or control peptide.
**In vivo quantification of invasive GFP-positive cells**

Infiltrated lungs (equal volume/weight between animals) were dissociated in Hank’s Balanced Salt Solution (HBSS) containing collagenase IV (0.6 Wünsch Units/mL) (Eurobio), dispase II (1mg/mL) (Sigma), DNase I (200 IU/mL) (Roche), CaCl$_2$ (75 µM), MgCl$_2$ (125 µM), during 1 hour at 37°C. The samples were then passed through a nylon filter (100 µm) (Dutscher) before to be centrifugated at 100g during 7 min. Pellets were resuspended in HBSS buffer containing DNAse I (200 IU/mL) and MgCl$_2$ (125 µM). Cell suspensions were centrifugated at 100 g during 7 min and resuspended in red blood cells lysis buffer (0.15 M NH$_4$Cl, 10 mM KHCO$_3$ 100 µM EDTA). After centrifugation at 100 g during 3 min, the cells were finally resuspended in HBSS buffer and quantified as invasive cells for their GFP expression among 200,000 cells analyzed by FACS with Cell Quest software (BD Biosciences Clontech).

**Analysis of DDB2 expression in breast cancer samples by quantitative real-time RT-PCR (qRT-PCR)**

Primary tumor samples were obtained, with informed consent, from 92 patients undergoing modified radical mastectomy for breast cancer, as a primary treatment without previous radiation or chemotherapy. A tumor fragment was taken near to the advancing edge of the primary tumor (avoiding its necrotic centre), frozen immediately, and stored in liquid nitrogen. The rest of the tumor was fixed in 6% buffer formaldehyde and embedded in paraffin for histological analysis. The TNM system of the UICC was used for tumor-node-metastasis staging (Sobin et al., 1997). Histological grading of the tumors was determined according to the Scarff, Bloom and Richardson method (SBR) (Bloom et al., 1957), with the lowest score of 1 (SBR1) for the well differentiated tumors, and with the highest score of 3 (SBR3) for the poorly differentiated tumors. Tumor samples were powdered in the frozen
state and cytosolic oestrogen (ER) and progesterone (PR) receptors were measured by dextran-coated charcoal assays with tritiated ligands (Thorpe et al., 1987).

Total RNA were isolated from 25-30 mg of frozen breast tumor tissue using the RNeasy Plus Universal Mini Kit (QIAGEN, France) according to the manufacturer's instructions. Total RNA (0.5 µg) were reverse transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad). One µL of diluted cDNA was analyzed with SyberGreen (Roche), in duplicate, using the LightCycler 480 real-time PCR system (Roche). The qRT-PCR data were analyzed using LightCycler® 480 software. Ct levels for DDB2 were normalized to the average Ct values of the internal controls Ubiquitin B (UBB) and RPLP0, whose primer sequences are done in Supplementary Table S1. Gene expression level graphs were drawn using a Box-and-Whisker plot. From the qRT-PCR data, differences between subgroups were evaluated statistically, using an ANOVA one-way approach and pairwise compared with a Student-Newman-Keuls, and were considered to be significant at a value of p<0.05.

References

Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer 1957;11:359-77.
