

Supporting Information

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SI Materials and Methods

Flow Cytometry. Confirmation of established MSC surface markers, such as CD90 and CD105, was carried out as described (1, 2). The anti-FPRL1 antibody (1:100) was purchased from LifeSpan Biosciences and rabbit IgG isotype control from Dako. These antibodies were detected with Alexa-488-conjugated goat anti-rabbit secondary antibodies (1:250; Molecular Probes). Analysis of MSC donor pools was performed on a BD FACSCalibur (BD Biosciences) by using BD CellQuest Pro software.

Boyden Chamber Migration Assay. Chemoattractants in medium containing 0.5% FBS were added to the lower compartment of a 48-well modified Boyden chamber in triplicate and overlaid with a gelatin-coated membrane (8 μm ; Neuro Probe). LL-37 (Innovagen) was added to a final concentration of 0.1, 1, or 10 $\mu\text{g}/\text{mL}$; EGF (R&D Systems) and phorbol myristate acetate (PMA) (Sigma) were both used at concentrations of 10 ng/mL . Serum-starved MSC (7.5×10^4) were added to the upper chamber. Where indicated, MSC were pretreated with 100 ng/mL pertussis toxin (Ptx) for 1 h then washed prior to loading in the chamber. LL-37 (1 $\mu\text{g}/\text{mL}$) and EGF were incubated with the anti-LL-37 neutralizing antibody (0.5 μg , clone 3D11; Hycult Biotechnology) or an IgG control (Chemicon/Millipore) for 1 h at 37 °C before loading. The remainder of the experiment was performed as described in ref. 3.

Invasion Assay. FluoroBlok inserts (8 μm ; BD Biosciences) were coated with growth factor-reduced Matrigel (BD Biosciences), and the experiment was conducted as described (2, 3). Chemoattractants were added in combination with 0.5% FBS. Serum-starved cells were seeded onto inserts at a density of 4.5×10^4 cells per insert. MSC were pretreated with Ptx and pre-incubation with the antibodies was employed in the exact manner as above. Relative fluorescent units were obtained with a FLUOstar Optima (BMG Labtech) fluorescent plate reader.

Proliferation Assay. MSC were seeded in 96-well plates (5000 cells per well) and allowed to adhere overnight. The next day, cells were washed with PBS and serum-free medium was added. After 24 h, cells were treated with 0, 1, 5, or 25 $\mu\text{g}/\text{mL}$ LL-37 or 10 ng/mL EGF in medium containing 0.5% FBS. Cells were analyzed every 24 h and the remainder of the experiment was performed as described in ref. 3.

Western Blot Analysis. Serum-starved MSC were treated with 5 $\mu\text{g}/\text{mL}$ LL-37 for the indicated time points before lysis and detection of proteins as described (1–3). ERK antibodies were purchased from Cell Signaling Technologies. β -actin was purchased from Sigma and secondary antibodies from Amersham. Quantification of band intensity was performed using National Institutes of Health ImageJ software (<http://rsbweb.nih.gov/ij/>).

In Vivo Migration Assay. Female SCID/CB17 mice ($n = 25$) were injected i.p. with 4×10^6 OVCAR-3 ovarian cancer cells (day 1), and tumors were allowed to establish for 3.5 weeks. After this time, half the mice were treated with 50 μg of nonspecific mouse IgG antibodies (Chemicon/Millipore) or 50 μg of anti-LL-37

antibodies (clone 3D11; Hycult Biotechnology). Antibodies were given twice a week for the duration of the experiment (days 26, 30, 33, 37, 40, 44, and 47). MSC were infected with 500 viral particles per cell of Ad-ffLUC-RDG, 24 h before injection of 5×10^5 cells on days 27, 34, 41, and 48 (4, 5). D-Luciferin (100 μL of 40 mg/mL in PBS; Caliper Life Sciences) was used to detect MSC as described, 7 d after MSC injections (4, 5). Bioluminescent images were acquired >3 min from anesthetized mice with the IVIS-Xenogen system (Caliper Life Sciences) and photons per second were measured in regions of interest by using Living Image software (Caliper Life Sciences).

Histology and IHC. After animals were killed, tumors were surgically removed, washed in PBS, and weighed. Tumors were fixed in formalin solution and embedded in paraffin. For histology of tumors, sections were stained with hematoxylin and eosin. Immunofluorescence staining was performed after reduction of autofluorescence by using 100 mM NH_4Cl for 10 min at room temp, antigen retrieval by boiling in 10 mM sodium citrate for 10 min, and blocking with 10% goat serum. The following antibodies were incubated with tissue sections for 1 h at room temp: mouse IgG1 (0.2 μg ; Chemicon); goat IgG (2 μg ; Dako); anti-LL-37 (0.2 μg or 1:50; clone 3D11; Hycult Biotechnology); anti-firefly luciferase (1:50; Promega, Madison, WI). The slides were washed with Tris-buffered saline containing 0.5% Tween-20 and incubated for 1 h at room temp with secondary antibodies (1:250; Alexa-568 or Alexa-488; Molecular Probes). Sections were washed again before addition of DAPI and ProLong Gold antifade reagent (Molecular Probes). IHC was performed as described by using Dako's Animal Research Kit (3). The anti-Ki-67 antibody (clone MIB-1; 1:100) was purchased from Dako. Images were evaluated by using a Zeiss Axioplan 2 fluorescence microscope and Intelligent Innovations software (SlideBook version 4).

LL-37 ELISA. Conditioned medium from MSC was collected 48 h after providing them with fresh medium. Hycult's LL-37 ELISA kit was used to measure hCAP-18/LL-37 according to the manufacturer's instructions. The experiment was repeated 3 times on 3 separate donor pools.

Analysis of MSC-Secreted Soluble Factors. MSC (5×10^4 in 24-well plates) were serum-starved for 24 h, then treated with 5 $\mu\text{g}/\text{mL}$ LL-37 in the presence of 0.5% FBS for 48 h. Conditioned medium was analyzed with Bio-Plex Cytokine Assays (Human Group I and II; Bio-Rad) following the manufacturer's instructions. Zymography assays were performed as before (3). Quantification of band intensity was performed by using ImageJ software. These experiments were performed at least 3 times on 3 separate MSC donor pools.

Tubule Formation Assay. Serum-starved HUVECs were resuspended in conditioned medium from untreated or LL-37-treated MSC then seeded onto Matrigel and assessed by microscopy every 2 h for 6 h. HUVECs were labeled with calcein AM as described in ref. 3. A similar method was used for MSC differentiation on Matrigel; however, in this case, cells were treated with 5 $\mu\text{g}/\text{mL}$ LL-37 or 10 ng/mL FGF2 (R&D Systems) in the presence of 0.5% FBS as reported earlier (2).

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