

# Hepatocyte growth factor promotes migration of human myeloma cells

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## ABSTRACT

Multiple myeloma is characterized by the accumulation and dissemination of malignant plasma cells in the bone marrow (BM). Cell migration is thought to be important for these events. We studied migration in a Transwell two-chamber assay and tested the mitogenic effect of various cytokines. In addition to insulin-like growth factor (IGF)-1 and stromal cell-derived growth factor (SDF)-1 $\alpha$ , previously known as chemoattractants for myeloma cells, we identified hepatocyte growth factor (HGF) as a potent attractant for myeloma cells. HGF-mediated migration was dependent on phosphatidylinositol-3-kinase (PI3K), involved the MAPK/Erk signaling cascade and VLA-4 integrins, but did not involve Akt, mTOR or G proteins.

Key words: myeloma, cell migration, HGF, SDF-1.

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## Design and Methods

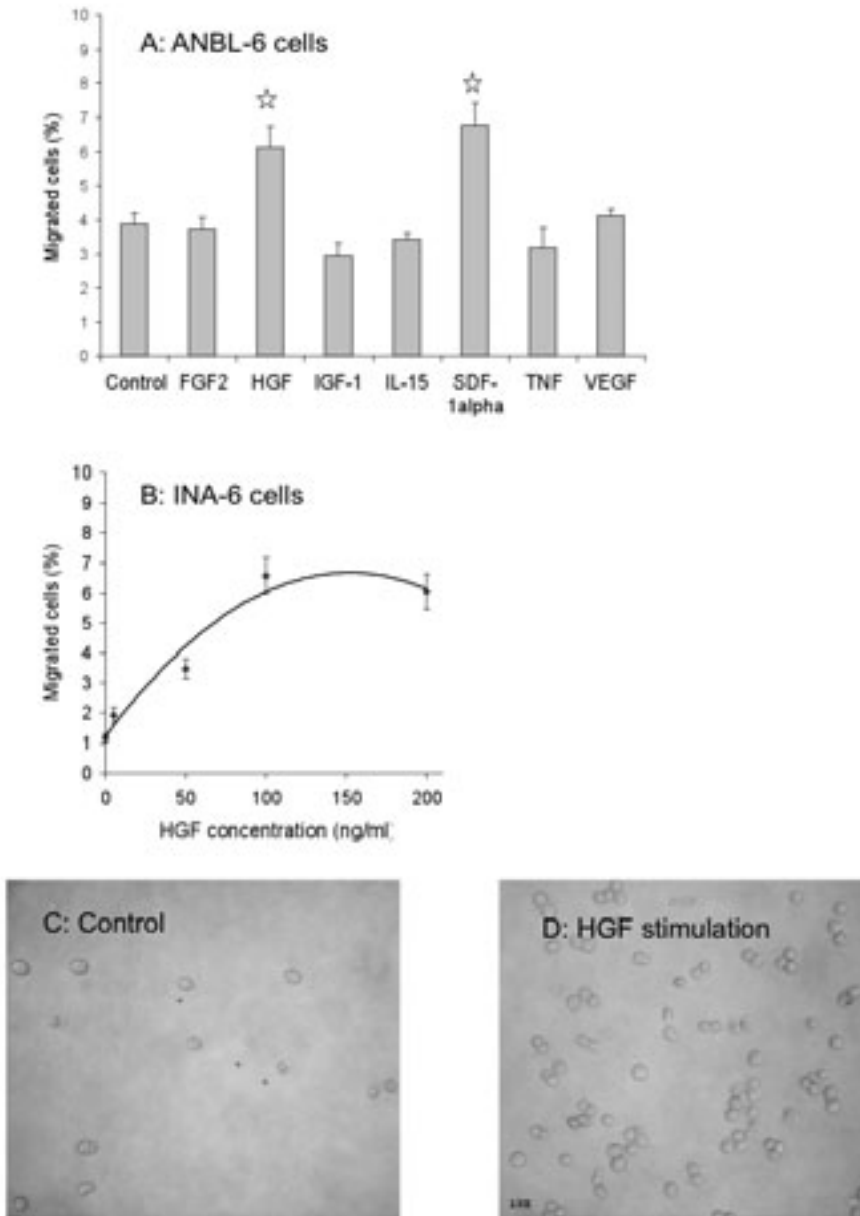
### Assessment of cell viability

Cells were washed in HBSS (37 °C) and seeded ( $3 \times 10^5$  cells/mL RPMI-medium) in 24-well culture plates (Life Technologies, Inc.) with HGF (150 ng/mL), with or without DMSO (0.2%) or inhibitors. Cells were incubated for 22 hrs. (5% CO<sub>2</sub>, 37 °C), washed in PBS with BSA(0.1%) and analyzed by the APOPTTEST-FITC-kit from Nexins Research (Kattendijke, The Netherlands). Percentage of apoptotic and live cells was calculated using a Coulter Epics XL-MCL flow cytometer (Beckton D) with EXPO32 ADC software.

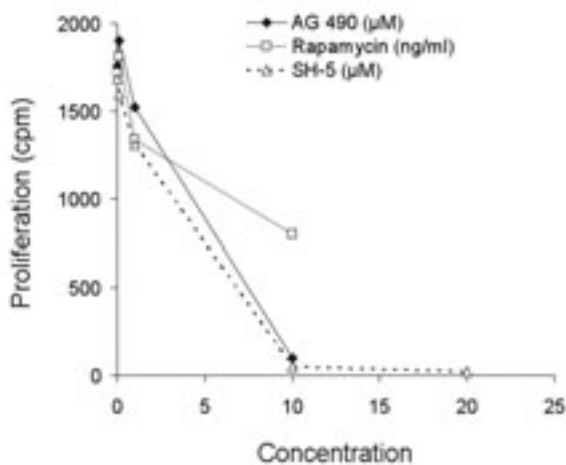
### Proliferation assay

Cells were seeded  $2 \times 10^4$  cells/well in 200  $\mu$ L of RPMI medium with HGF (150 ng/mL) in 96-well culture plates (Costar, Corning, NY). Inhibitors were added as indicated for each experiment. After 54 hrs., the cells were pulsed with 0.75  $\mu$ Ci methyl-<sup>3</sup>H-thymidine (NEN Life Science Products, Boston, MA) per well and harvested 18 hrs. later with a Micromate 96 well harvester, and beta radiation was measured with a Matrix 96 counter (Packard, Meriden, CT, USA).

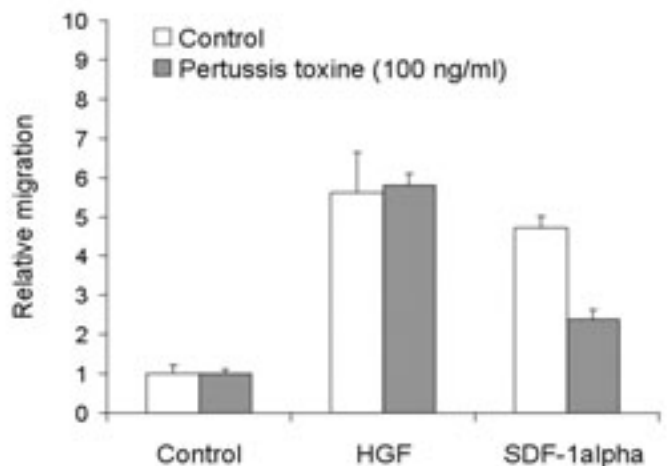
## Supplementary Figure



**Figure S1.** ANBL-6 cells (A) or INA-6 cells (B) were seeded in the upper compartment in a two-chamber Transwell migration assay. To the bottom compartment were added RPMI-medium with or without FGF2 (10 ng/mL), HGF (150 ng/mL), IGF-1 (100 ng/mL), IL-15 (20 ng/mL), SDF-1 $\alpha$  (75 ng/mL), TNF (20 ng/mL) or VEGF (100 ng/mL), or indicated concentrations of HGF. After 22 hours at 37 °C, cells in the bottom compartment were counted and percentage of migrated cells calculated. Error bars represent standard deviation of 3 repeated counts in two independent measurements. Representatives for 3 similar experiments are shown. Asterisks indicate significantly increase in migration according to an unpaired Student's two-tailed test, \*( $p < 0.01$ ). Migrated INA-6 cells were photographed at 10x10 magnification at control (C) and when HGF was added to the lower chamber (D).



**Figure S2.** The efficacy of SH-5, rapamycin and AG490 was proven by their influence on INA-6 cell proliferation when cells were grown in RPMI-medium with HGF (150 ng/mL). SH-5 (10  $\mu$ M) fully inhibited proliferation. Rapamycin (5 ng/mL) and AG490 (5  $\mu$ M) reduced proliferation by 65% and 85% respectively. Error bars represents  $\pm$  1SD of three repeated counts in three independent measurements. The figures show one representative out of three similar experiments.



**Figure S3.** The effect of inhibiting G protein-coupled receptors was assessed. INA-6 cells were stimulated with or without HGF (150 ng/ml) or SDF-1 $\alpha$  (75 ng/mL) and analyzed by the two-chamber migration method as described in Design and Methods. Pertussis toxin was added to both chambers. The result shown represents one out of four similar experiments, and error bars represent  $\pm$  1 SD of six parallel measurements.