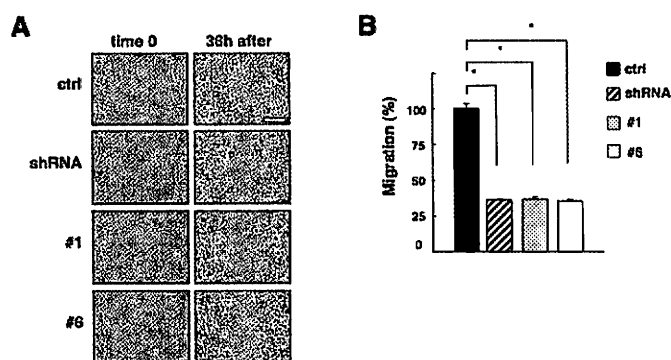


## The scaffold protein c-Jun NH<sub>2</sub>-terminal kinase-associated leucine zipper protein regulates cell migration through interaction with the G protein G<sub>α13</sub>

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Scaffold proteins for MAP kinase (MAPK) signalling modules play an important role in the specific and efficient signal transduction of the relevant MAPK cascades. Here, we investigated the function of the scaffolding protein c-Jun NH<sub>2</sub>-terminal kinase (JNK)-associated leucine zipper protein (JLP) by depleting it in cultured cells using a short hairpin RNA (shRNA) against human JLP. HeLa and DLD-1 cells stably expressing the shRNA showed a defect in cell migration. The re-expression of full-length shRNA-resistant mouse JLP rescued the impaired cell migration of the JLP-depleted HeLa cells; whereas, a C-terminal deletion mutant of mouse JLP, which failed to bind the G protein G<sub>α13</sub>, showed little or no effect on the cell migration defect. Furthermore, although a constitutively active G<sub>α13</sub> enhanced the migration of control HeLa cells, the G<sub>α13</sub>-induced cell migration was significantly suppressed in the JLP-depleted HeLa cells. Taken together, these results suggest that JLP regulates cell migration through an interaction with G<sub>α13</sub>.



**Figure Impairment of cell migration in JLP KD HeLa cells.** (A) The control (ctrl) HeLa cells, the pooled JLP KD HeLa cells, and clones #1 and #6 stably expressing JLP-shRNA1 were replated onto collagen-coated dishes at a saturation density. After being scratched, the wounded monolayers were allowed to heal for 36 h. Scale bar, 200  $\mu$ m. Experiments were performed independently three times, and representative results are shown. (B) The cell migration activity of these cell lines was quantified by a Transwell assay. Values represent the number of migrated pooled KD, clone #1, and clone #6 cells as a percentage of the number of control cells (means  $\pm$  SD from three independent experiments, \* $p$  < 0.01, Student's  $t$ -test).

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