The distinct roles of Ras and Rac in PI 3-kinase-dependent protrusion during EGF-stimulated cell migration

JCS005298 Supplementary Material

Files in this Data Supplement:

- **Supplemental Figure S1** -

  Fig. S1. Immunofluorescence staining controls. (A) MTLn3 cells were stimulated with EGF for 1 minute, fixed and stained with anti-Rac antibodies. In the right panels, antibodies were preincubated with recombinant GST-Rac. (B) MTLn3 cells were transfected with control (luciferase, left panel) or Rac1 (right panel) siRNA. The cells were fixed and stained with anti-Rac antibodies. (C) MTLn3 cells were stimulated with EGF for 1 minute, fixed and stained with GST-CRIB followed by anti-GST antibodies. In the right panel, anti-GST antibodies were preincubated with recombinant GST. (D) MTLn3 cells were transfected with control (luciferase, top panels) or K-Ras and N-Ras (right panel) siRNA. The cells were stimulated with EGF for 0 or 3 minutes, fixed and stained with GST-CRIB followed by anti-GST antibodies.

- **Supplemental Figure S2** -

  Fig. S2. Rac and Ras knockdowns. (A) Anti-Rac blot of lysates from control siRNA or Rac1 siRNA-treated cells. (Upper panel) Cells treated with a single Rac1 siRNA oligonucleotide (in triplicate). (Lower panel) Cells treated with a pool of four Rac siRNA oligonucleotides (in duplicate). (B) Ras knockdown. (Left panel) Relative mRNA levels of K-Ras, N-Ras and H-Ras in control MTLn3 cells, determined by Q-RT-PCR. Data is the mean ± s.e.m. of three determinations. (Right panel) Knockdown of K-Ras, N-Ras and H-Ras message in siRNA-treated cells. Data represents the fraction of initial expression remaining, and is the mean ± s.e.m. of four determinations.

- **Supplemental Figure S3** -

  Fig. S3. EGF-stimulated protrusion in Rac1 knockdown cells. MTLn3 cells were transfected with control (luciferase) siRNA or a pool of four Rac1 siRNA oligonucleotides. Protrusion was measured as in Fig. 4; the magnitude of the cell areas is larger because the images were collected with a 40× objective using a different camera.

- **Supplemental Figure S4** -

  Fig. S4. Protrusion in cells treated with a Rac inhibitor. (A) MTLn3 cells were treated without or with varying doses of NSC23766, and were incubated in the absence or presence of EGF or 5% serum as indicated. The cells were lysed and GST-CRIB pull-downs were blotted with anti-Rac antibody as described. (B) MTLn3 cells were treated without or with NSC23766 and protrusion was measured as in Fig. 4; the magnitude of the cell areas is larger because the images were collected with a 40× objective using a different camera.

- **Movie 1** -

  Movie 1. Time-lapse images of control and Rac siRNA-treated cells stimulated with EGF. The time-lapse duration is 10 minutes, and images were captured at 10-second intervals. (Left panel) Control. (Right panel) Rac siRNA.

- **Movie 2** -

  Movie 2. Time-lapse images of control and Rac1 siRNA-treated cells in 5% serum media. The time-lapse duration is 1 hour, and the images were captured at 1-minute intervals. (Left panel) Control. (Right panel) Rac1 siRNA.