Inside-out signaling through FAK–integrin axis may regulate circulating cancer cell metastatic adhesion

Christina Downey-Biechler[a], David H. Craig[b], Shyam K. More[b], and Marc D. Basson[b,1]

In PNAS, Chang et al. (1) call attention to the function of focal adhesion kinase (FAK) in mediating inside-out signaling regulating immune cell adhesion to the endothelium by elegantly studying the FAK–RAP1–RIAM–talin pathway for feedforward inside-out signaling through integrin described by Lagarrigue (2). Chang et al. solve the crystal structure of the RIAM inhibitory segment (IN), RAS association (RA), and Pleckstrin homology (PH) domains, showing that IN/RA-PH domain interaction autoinhibits RIAM, hampering RIAM–RAP1 association. This abrogates RAP1-dependent translocation of RIAM to the plasma membrane. Upon T cell receptor activation, FAK phosphorylates Tyr45 on the RIAM IN domain, abolishing the RIAM autoinhibitory loop and facilitating RIAM membrane translocation (1).

However, FAK-driven inside-out signaling also regulates epithelial cell adhesion (3). In particular, Akt1–FAK interaction also mediates adhesion of epithelial cancer cells to matrix proteins (4–6) and endothelial cells (7) exposed to forces like pressure (4, 7) and shear (8). Activation of this pathway impairs survival in animal models of direct surgical tumor implantation (9), but Chang et al.'s study raises questions of whether circulatory pressure and shear forces activate circulating cancer cell adhesion and metastasis in vivo.

We treated luciferase-expressing murine CT-26 colon cancer cells with vehicle or FAK or Akt1 inhibitors, at ambient or 15-mmHg increased pressure for 30 min. We injected them into the tail vein or portal circulation of BALB/c mice, evaluating signaling and integrin affinity by flow cytometry, metastasis by imaging and postmortem organ weight, and survival. When CT-26 cells were recovered by cardiac puncture 30-min post-tail vein injection, FAK-Tyr-397 phosphorylation, Akt-Ser-473 phosphorylation, and 9EG7–β1-integrin–epitope activation reflecting integrin heterodimer binding affinity were increased (Fig. 1*A*), suggesting that systemic circulatory forces activate FAK, Akt, and β1-integrin heterodimer binding affinity in circulating cancer cells.

These signals impact tumor cell adhesion and metastasis. CT-26-Luc cells preincubated at ambient or 15-mmHg increased pressure for 30 min were injected into the low-pressure portal circulation via the spleen in BALB/c mice. Pressure-activated cells increased subsequent tumor size by imaging (Fig. 1 *B* and *C*) and spleen and liver weights (Fig. 1*D*) vs. control cells. Inhibiting CT-26-Luc FAK or Akt before injection decreased subsequent tumor burden without pressure preactivation and prevented the pressure increase in tumor burden (Fig. 1 *B* and *C*). Indeed, initial pressure activation significantly impaired subsequent survival, which was blocked by FAK or AKT inhibitor pretreatment (Fig. 1*E*). Portal pressures are low and transit time to the liver after injection rapid. We further hypothesized that activation of the Akt–FAK–β1-integrin pathway by exposure to the high-pressure/shear systemic circulation would obscure the pressure pretreatment effect. Pressure pretreatment before tail vein injection did not further alter tumor implantation in the lung (Fig. 1 *F* and *G*) or long-term survival (Fig. 1*H*). However, FAK or Akt inhibitor pretreatment before tail vein injection reduced lung metastasis and improved survival (Fig. 1 *G* and *H*).

FAK inside-out signaling may activate not only leukocyte adhesion but also cancer metastasis. Future studies may investigate whether RIAM mediates force-activated cancer cell adhesion and how Akt1 influences leukocyte adhesion.

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[a]Department of Family Medicine, Ascension Hospital System Providence, Southfield, MI 48075; and [b]Department of Surgery, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58202

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Conflict of interest statement: M.D.B. is coinventor of a pending patent application on the use of small molecules to inhibit FAK–Akt interaction to prevent cancer metastasis that has been jointly filed by the University of North Dakota and Michigan State University. No data from that patent application are presented here, however, and the FAK and Akt inhibitors used here are commercially available and not covered in that patent application. The authors have no other potential conflict of interest.

[1]To whom correspondence may be addressed. Email: marc.basson@und.edu.

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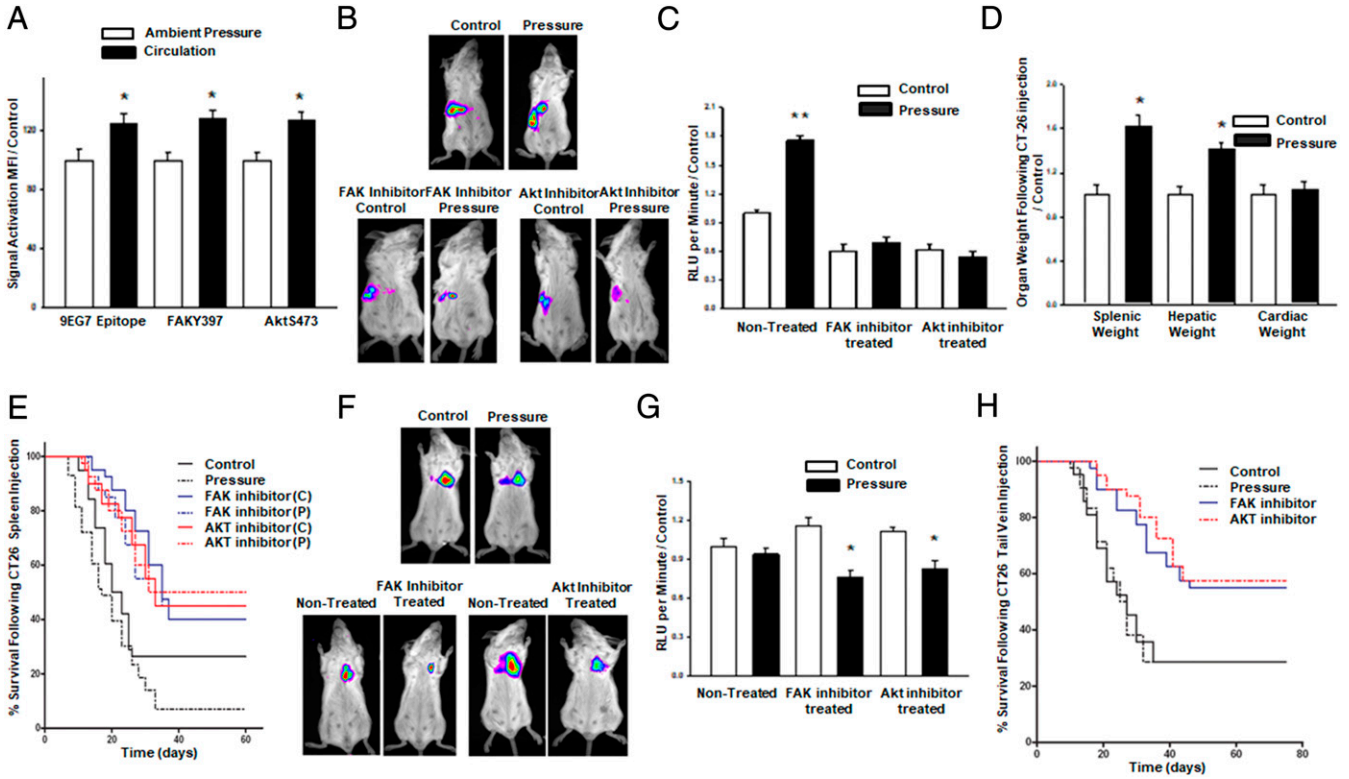

Fig. 1. Force-activated signaling in metastasis. (*A*) Flow cytometry for 9EG7, FAKY397, and AktS473 in CSFE-stained CT-26 cells from cardiac puncture 30 min after BALB/c tail vein injection vs. uninjected controls ($n = 6$; $^*P < 0.05$). (*B*) After preincubation with or without 15-mmHg increased pressure, with or without 20 μM FAK inhibitor Y15 or 1 μM Akt inhibitor IV, 750,000 CT-26-Luciferase cells were injected via BALB/c spleens. Typical pseudocolored bioluminescence (pink–red, low–high) at day 7. (*C*) Quantitation for *B* ($n = 16$; $^{**}P < 0.01$ vs. nontreated control). (*D*) Splenic and hepatic weights ($n = 16$; $^*P < 0.05$). (*E*) Survival of mice treated as in *B* [$n = 20$; $P < 0.05$ pressure vs. control, FAK inhibitor (P) or Akt inhibitor (P)]. (*F*) Typical pseudocolored images 7 d after 10^7 luciferase-expressing CT-26 cells pretreated with or without 15-mmHg pressure, with or without Y15 or Akt inhibitor, were injected into BALB/c mouse tail veins. (*G*) Quantitation for *F* ($n = 20$; $^*P < 0.05$). (*H*) Survival as in *F* ($n = 20$ control–pressure, $n = 12$ for inhibitors; $P < 0.05$ control or pressure vs. each with FAK or Akt inhibitors).

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