Pictures in Molecular Medicine

Three-dimensional visualization of intravascular tumor cells in mice

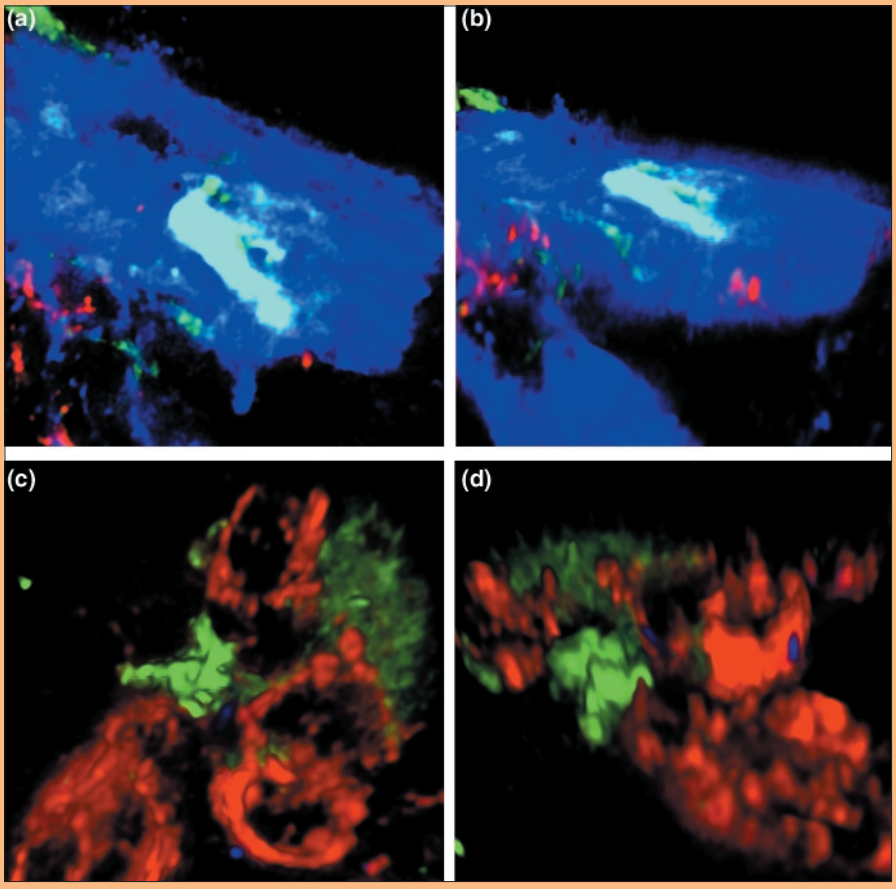

Fig. 1. (a) In a control mouse with no injected heparin, a cancer cell (green fluorescence) has a dense cloak of platelets (blue fluorescence), hence the green cancer cell appears light blue. (b) An image of the same cell from a different perspective. (c) In a mouse injected with heparin, a marked reduction of blue platelets around green tumor cells was seen, which allows an increased association of red stained monocytes (macrophage precursors) with the tumor cell. (d) same image as (c) from a different perspective.

Hematogeneous metastasis of cancer is a cascade of events involving intravasation of tumor cells into the bloodstream, evasion of innate immune surveillance, adhesion to vascular endothelial cells of distant organs, with extravasation and colonization of tissues. During this process tumor cells form complexes with blood platelets and leukocytes, and such emboli appear to contribute to arrest in the vasculature. Although the formation of tumor cell-platelet aggregates was well known to promote metastasis, the mechanism of the interaction was unclear.

Selectins are vascular receptors that can mediate interactions of tumor cells with platelets, leukocytes and endothelium *in vitro*, suggesting their possible involvement in metastasis[1]. The ability of platelet P-selectin to bind monocytes and induce release of a tissue factor makes it a candidate to explain the platelet facilitation of metastasis. Indeed, P-selectin deficiency attenuated metastatic spread of human carcinomas in a mouse model[2].

Studies in mice and humans have suggested that heparin administration can reduce cancer metastasis. We found that heparin is a potent inhibitor of P- and L-selectin at concentrations achievable in patients[3]. A single injection of heparin attenuates immediate platelet–tumor cell interactions, with a marked reduction in metastasis[4] within six weeks underlining the impact of blocking early steps in the metastatic cascade. To visualize the effects of heparin treatment, lung sections from mice injected with the calcein-labeled tumor cells (green) were stained with AMCA-labeled anti-CD41 for platelets (blue) and Cy3-labelled anti-Mac-1 for monocytes (red) and analyzed by deconvolutional microscopy[4]. Viewing of these tumor cell–platelet–leukocyte complexes was enhanced by a three-dimensional computer reconstruction and generation of animated 'fly-by' movies of the images[4], (available online at http://www.pnas.org/cgi/content/full/98/6/3352/DC1). Still images from these reconstructions are presented in Fig. 1. Thus, heparin inhibits platelet–tumor cell interactions at least partly via blockade of P-selectin. These findings also underline the importance of a 'platelet cloak' not only for assisting tumor cells to initially lodge in blood vessels, but also possibly for protecting them from potentially cytotoxic effector cells in the vasculature, thus permitting subsequent metastatic progression.

References

1 Kim, Y.J. *et al.* (1999) Distinct binding sites for the three selectins can mediate pathological interactions amongst blood and vascular cells. *Am. J. Pathol.* 155, 461–472
2 Kim, Y.J. *et al.* (1998) P-selectin attenuates tumor growth and metastasis. *Proc. Natl. Acad. Sci. U. S. A.* 95, 9325–9330
3 Koenig, A. *et al.* (1998) Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins – Implications for the use of unfractionated and low molecular heparins as therapeutic agents. *J. Clin. Invest.* 101, 877–889
4 Borsig, L. *et al.* (2001) Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3352–3357

Lubor Borsig
Richard Wong
James Feramisco
David R. Nadeau†
Nissi M. Varki
Ajit Varki*
Glycobiology Research and Training Center, The Cancer Center, Depts of Medicine and Cellular and Molecular Medicine and †The San Diego Supercomputer Center, University of California, San Diego, La Jolla, CA 92093-0687, USA.
*e-mail: avarki@ucsd.edu

 1471-4914/01/$ – see front matter PII: S1471-4914(01)02094-9