Adding Complexity to Fibronectin-Platelet Interactions

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The article by Chauhan et al in the current issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* adds a new complexity to the roles of fibronectin in formation of hemostatic plugs and thrombotic occlusions. Normal mice, in which >99.5% of plasma fibronectin lacks the alternatively spliced EDA domain, were compared with mice genetically engineered to have only EDA-containing (EDA⁺) fibronectin in the circulation. Mice with only EDA⁺ fibronectin developed occlusive platelet thrombi more quickly in arterioles damaged by ferric chloride and suffered increased mortality from pulmonary emboli after an infusion of collagen-coated chamber, surface coverage by adherent platelets was greater in the mice with EDA⁺ fibronectin. Examination of the photomicrographs suggests that the volume of platelet thrombi in the collagen-coated chamber also was greater after perfusion of blood from mice with EDA⁺ fibronectin. Three different read-outs, therefore, indicate that thrombus build-up is enhanced by the presence of EDA⁺ fibronectin.

The increase in susceptibility to thrombi is even more remarkable when one considers concentrations of plasma fibronectin. Mice with 50% of normal plasma fibronectin have been shown previously to be protected from occlusive thrombi after arteriolar injury when compared with mice with 100% levels. Flow chamber experiments suggest that fibronectin concentrations below 50% normal are even more protective against thrombosis. The concentration of fibronectin in plasma of mice expressing the EDA⁺ variant was only 20% of normal. Thus, a concentration that might be expected to be highly protective against thrombus build-up instead enhances thrombus formation.

Several mechanisms are suggested for why EDA⁺ fibronectin enhances build-up of platelet thrombi. EDA is a type III module inserted amid other type III modules (see Figure 1A of Chauhan et al) and thus may function simply as another, albeit more exotic, type III module in a conga line of type III modules. However, because the known receptors for EDA, α4β1 and α9β1 integrins, are not present on platelets, the authors favor mechanisms whereby the insertion of EDA changes the dynamics of the various parts of fibronectin in relation to one another, akin to adding an extra dancer to a Busby Berkeley musical production. EDA⁺ fibronectin may be assembled more readily by activated platelets, a step that is essential for the activity of fibronectin in stabilizing platelet thrombi, and thus form a scaffold that interacts with αIIbβ3 integrin and other platelet receptors.

Mice with only EDA⁺ fibronectin have a shorter lifespan than normal mice for reasons that have not been apparent in postmortem examinations. Interestingly, mice unable to make EDA⁺ fibronectin (which, it should be said, also have a shorter lifespan that may be attributable to skin problems) develop less atherosclerosis than normal mice with regulated splicing of the EDA domain. Diabetes, acute stroke, and other pathological conditions in humans, as described by Chauhan et al, have been associated with a several-fold increase in EDA⁺ fibronectin in plasma. It is suggested that an elevated level of EDA⁺ plasma fibronectin is a risk factor for thrombosis.

The authors appropriately question the suitability of mice with 100% EDA⁺ plasma fibronectin as models for pathological conditions in which only 1% to 3% of plasma fibronectin is EDA⁺. The EDA⁺ fibronectin in plasma of patients with the pathological conditions likely comes from diverse cells and platelets stimulated by the conditions to secrete fibronectin. Most plasma fibronectin, in contrast, comes from hepatocytes. Hepatocytes normally splice fibronectin mRNA to exclude EDA completely and also partially exclude the nucleotides encoding the adjacent IIICS region (see Figure 1A). Fibronectin is secreted as a disulfide-bonded dimer. Probably because of instability in the secretory pathway of dimers in which both subunits lack IIICS, almost all plasma fibronectin secreted by hepatocytes is a heterodimer in which one subunit contains IIICS and one does not. In contrast, IIICS is present in most subunits of EDA⁺ “cellular” fibronectin. The EDA⁺ mouse was produced by optimizing the splice sites surrounding the exon encoding EDA, leaving the IIICS region to be spliced in its normal regulated fashion. Therefore, the hepatocyte of the EDA⁺ mouse is challenged to make fibronectin dimers from subunits with which it may be ill-equipped to handle; all are EDA⁺ rather than EDA⁺, and some are unusual in being EDA⁺IIICS⁺ rather than EDA⁺IIICS⁺ as in “cellular” fibronectin. Instability of such dimers during secretory processing within the hepatocyte may account for the low plasma concentration of fibronectin in the EDA⁺ mouse. It will be interesting to study EDA⁺ mice, which should have more normal plasma fibronectin concentrations that the EDA⁺ mouse and a mix of dimers created by the permutations of subunits that are EDA⁺IIICS⁺, EDA⁺IIICS⁻, EDA⁺IIICS⁻, and EDA⁺IIICS⁻. Such mice may mimic better the complexity of circulating fibronectin dimers found in human diseases.
Disclosures
None.

References
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