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In conclusion, these innovative and very well conducted studies represent a major achievement in the field of hematologic malignancies. They bring miRNAs nearly from the bench to the bedside because they provide the preclinical evidence for the development of novel miRNA-based prognostic and therapeutic options in WM.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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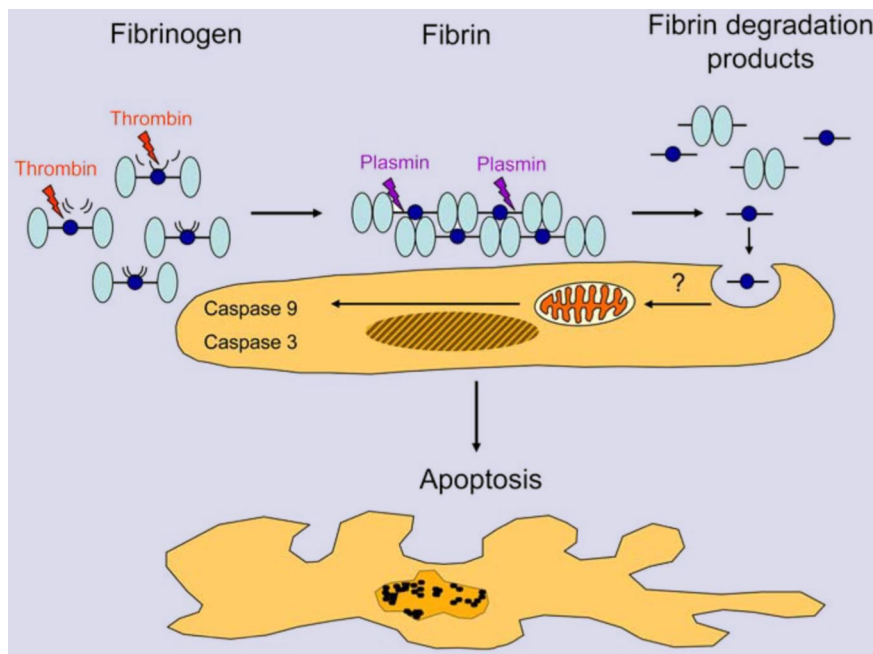
## ● ● ● THROMBOSIS & HEMOSTASIS

Comment on Guo et al, page 4431

# How can fibrinolysis induce cell death?

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In this issue of *Blood*, Guo and colleagues elucidate molecular details of a novel mechanism, linking the degradation of fibrin (but not fibrinogen) by plasmin to apoptosis of placental trophoblast cells.



Fibrin is formed by the action of thrombin on fibrinogen releasing fibrinopeptides A and B from the A $\alpha$ - and B $\beta$ -chains of fibrinogen. Plasmin generated by the plasminogen activators uPA or tPA cleaves fibrin into fibrin degradation products (shown here: D-dimer, light blue, and fragment E, dark blue). Fibrin fragment E is internalized by cells and induces apoptosis of trophoblast cells. Illustration by Thomas Nardelli.

There is convincing evidence from the literature that disorders of the maternal coagulation system are associated with complications of pregnancy including fetal loss. In many studies, an association between fibrin deposition in the placenta and apoptosis of trophoblast cells has been observed.<sup>1</sup> The group of Weiler and collaborators have shown previously that mouse embryos deficient in the thrombin receptor thrombomodulin (*Thbd*<sup>-/-</sup>) die before the development of a functional cardiovascular system because of a defect in the placenta.<sup>2</sup> In a more recent study that analyzed the mechanisms responsible for the placental defect in these mice, they found that activated coagulation factors induce growth inhibition and apoptosis of placental trophoblast cells.<sup>3</sup> While the growth-inhibiting effect can be attributed to the activation of protease-activated receptors, cell death is caused by degradation products of fibrin. Neither fibrinogen nor intact fibrin nor degradation products of fibrinogen caused apoptosis.

In this issue of *Blood*, Guo et al perform a study based on these previous findings that discloses the structural requirements and molecular mechanisms involved in fibrin degradation product-mediated cell death.<sup>4</sup> They show that the apoptosis-inducing activity is not restricted to the mouse trophoblast system but is also seen with human fibrin degradation products and a variety of cell types. They can furthermore assign the apoptosis-inducing activity to a sequence within the A $\alpha$ -chain of fibrin fragment E, which has to be cleaved by thrombin as well as by plasmin to gain apoptosis-inducing activity. Part of the proapoptotic activity can be attributed to an RGD-motif, but the majority is RGD-independent. Induction of apoptosis by fibrin fragment E requires its uptake by the cell. Uptake, but not apoptosis, is mediated via a motif located within the sequence A $\alpha$ 52-81. Apoptosis-inducing activity itself is located in A $\alpha$ 17-37. The internalization of fibrin fragment E, but not the intracellular mechanism mediating apoptosis, is caveolin-1-dependent. Intracellular pathways have not yet been analyzed in detail, but data presented suggest activation of the mitochondrial pathway and involvement of caspases 9 and 3.

This newly described pathway linking fibrin degradation to apoptosis may play a role in several physiologic and pathophysiologic situations. It may lead to trophoblast cell death, causing placental insufficiency and

pregnancy loss in situations of increased fibrin deposition and degradation. Local fibrin deposition and fibrinolysis are found at sites of wound healing and tissue remodeling. At these sites, the induction of apoptosis by fibrin degradation products could facilitate the clearance of damaged cells. Cross-linked fibrin is also a component of the matrix of most cancers,<sup>5</sup> and degradation of fibrin by plasmin generated in the vicinity of tumor cells could support tumor cell apoptosis. The work of Guo et al, which focuses mainly on structural requirements of the proapoptotic molecule, not only raises many new questions related to the intracellular pathway—mediating apoptosis but also represents the basis for many new studies analyzing the physiologic relevance of apoptosis induction by fibrin degradation products.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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mors in epithelia with a preference for induction of mammary gland adenocarcinomas. This interesting and unexpected finding may limit the therapeutic use of VEGF-D. Therapeutic intervention by neutralization of VEGF-D for diseases characterized by excessive angiogenesis, such as cancer, may therefore be a more promising approach. Indeed, inhibition of metastatic spread, microhemorrhage, and collapse of tumor vessels has been described in mice injected with blocking monoclonal antibodies<sup>5</sup> or soluble VEGFR-3.<sup>6</sup>

Although it was reported before that VEGF-D can facilitate tumor growth and support metastasis formation, probably due to enhanced vessel/tumor cell interaction surface, it is very interesting to question how VEGF-D can give rise to spontaneous tumors. It suggests that either the growth factor itself can transform normal epithelial cells into tumor cells—a hypothesis that is currently considered unlikely—or that normal epithelial cells are continuously challenged by genetic or epigenetic alterations, having more chance to result in tumor cells (normally a very rare event) when a more angiogenic environment is available. In the latter situation, tumor growth may be supported by better supply of oxygen and nutrients, by enhanced vascular permeability, a beneficial cytokine/chemokine milieu, or by a changed adhesion molecule profile. Another possibility is the VEGF-D-mediated generation of an immune-privileged situation. Indeed, VEGF-D has been reported to contribute, in a concerted action with other angiogenic growth factors, to endothelial cell anergy and subsequent escape from immune surveillance.<sup>7</sup> All these conditions are supportive for an anti-VEGF-D strategy to intervene with tumor growth. Such an approach would potentially have the same problems as current anti-VEGF strategies in the clinic,<sup>8</sup> such as induction of drug-induced resistance, but this may be overcome by combining different strategies of therapy. The paper by Kärkkäinen et al definitely underscores the importance of VEGF-D in the regulation of angiogenesis and suggests an opportunity for the development of new and innovative medical technology for cancer and other human diseases.

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#### ● ● ● VASCULAR BIOLOGY

Comment on Kärkkäinen et al, page 4468

## Lymphangiogenesis factors: a target for therapy?

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In this issue of *Blood*, Kärkkäinen and colleagues demonstrate, using a VEGF-D transgenic mouse model, that overexpression of human VEGF-D induces a proangiogenic phenotype, increases regeneration after ischemic injury, and also induces the formation of tumors.

Vascular endothelial cell growth factor D (VEGF-D) was identified in the mid-1990s as a c-fos-induced growth factor sharing structural and functional characteristics with VEGF-C and displaying lymphangiogenic properties through activation of the lymph vasculature receptor VEGFR-3.<sup>1,2</sup> In humans, VEGF-D binds to VEGFR-3 as well as to VEGFR-2, of which the latter is the receptor for VEGF-transducing signals leading to formation of blood vessels. The study by Kärkkäinen et al, which reports the generation of human VEGF-D transgenic mice using a method based on perivitelline oocyte injection of a lentiviral vector expressing the growth factor, provides new insight into the biology of VEGF-D.<sup>3</sup> The transgenic mice, constitutively expressing VEGF-D in many organs, clearly demonstrate a proangiogenic phenotype with enhanced blood vessel capillary density in muscle tissue while a lack of increased numbers of lymphatic capillaries suggest no

effect on lymphangiogenesis. The enhanced healing capacity of transgenic mice after ischemic injury was also suggested to be mainly due to VEGFR-2 signaling and enhanced blood vessel formation. These results are consistent with earlier studies where knockout approaches were used, demonstrating that VEGF-D does not have a major lymphangiogenesis function.<sup>4</sup> This study by Kärkkäinen et al, as well as studies by others, may suggest the potential for growth factor therapy of ischemic diseases. In addition, discussions on the use of therapeutic lymphangiogenesis for diseases with associated lymphedema after surgery or radiotherapy are ongoing. Although this is an attractive approach, (lymph-)angiogenic growth factors can enhance vascular permeability, which may pose an additional problem.

In the present study, it was observed that mice with human VEGF-D incorporated in their genome developed spontaneous tu-