# A DNA-unraveling enzyme in neutrophils essential for deep vein thrombosis

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(Medical Xpress)—It takes more than platelets, thrombin and fibrin to build a deep vein thrombosis (DVT). Increasingly, researchers are recognizing that neutrophils—cells better known for their role in immune defense—play an active role in DVT formation by releasing platelet-catching nets made of chromatin, a tightly-wound mix of DNA and associated proteins.

Now a team of researchers from Boston Children's Hospital's Program in Cellular and Molecular Medicine report that mice lacking a neutrophil enzyme called PAD4, which helps unravel the chromatin in neutrophils' nuclei, cannot form DVTs. Their work is a first step toward developing safer, more targeted treatments for DVTs.

The team, led by graduate student Kimberly Martinod and senior investigator Denisa Wagner, PhD, published their findings the week of May 6 in the online Early Edition of the *Proceedings of the National Academy of Science*.

DVTs are blood clots that form within veins deep inside the body, usually in the lower leg or thigh. They are largely associated with lack of motion, for instance if a person is bedridden or sitting still for a long period of time on a flight. If a DVT breaks free, it travels through the blood stream and can become lodged in the lung, causing a potentially fatal pulmonary embolism.

In recent years, the Wagner laboratory and other groups have started to

build a body of work on the involvement of neutrophils in DVT formation. When activated, neutrophils can release a mesh of chromatin called a neutrophil extracellular trap (NET). NETs help defend against infection, but in 2010 Wagner's laboratory showed they also act as a scaffold on which platelets can aggregate to form DVTs.

"It has long been thought that clot formation in DVTs relies solely on mechanisms that drive coagulation through thrombin and fibrin, which is why current treatments for DVTs focus on prevention or reversal of coagulation with agents like low molecular weight-heparin," said Wagner. "But in animal models, we can reduce DVT formation by depleting neutrophils or by treating with enzymes that cleave DNA."

The means by which neutrophils unravel (or decondense) and expel their chromatin to produce NETs has not yet been fully characterized. In this new paper, Wagner, Martinod and their collaborator Yanming Wang, PhD, at Pennsylvania State University asked whether PAD4, an enzyme that modifies histones in a way that promotes unraveling of chromatin, is required for NET and DVT formation.

Their research showed that neutrophils from mice lacking PAD4 could not produce NETs, and that such mice were nearly completely protected from DVT formation. Giving them bone marrow neutrophils from mice with active PAD4 genes eliminated that protection.

"Neutrophils without PAD4 could not form NETs no matter how we stimulated them," according to Wagner. "And 90 percent of PAD4-knockout mice were unable to form DVTs despite the presence of all of the other known features needed for DVT formation."

Concerned about potential side effects, the researchers found that knocking out PAD4 did not affect the formation of blood clots in response to injury. "Bleeding times in both wild type and PAD4

knockout mice were similar," Wagner noted. "There does not appear to be any effect of inhibiting PAD4 on blood clotting in response to injury."

Wagner and her colleagues believe the study adds to a body of evidence pointing to NETs and the pathways that produce them as potential therapeutic targets for DVTs.

"We may have an opportunity here to create a new therapeutic approach for cases of DVT or pulmonary embolism, or one that targets both coagulation and neutrophils to provide better outcomes."

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