Researchers describe regulatory protein controlling the patterning of the lymphatic system

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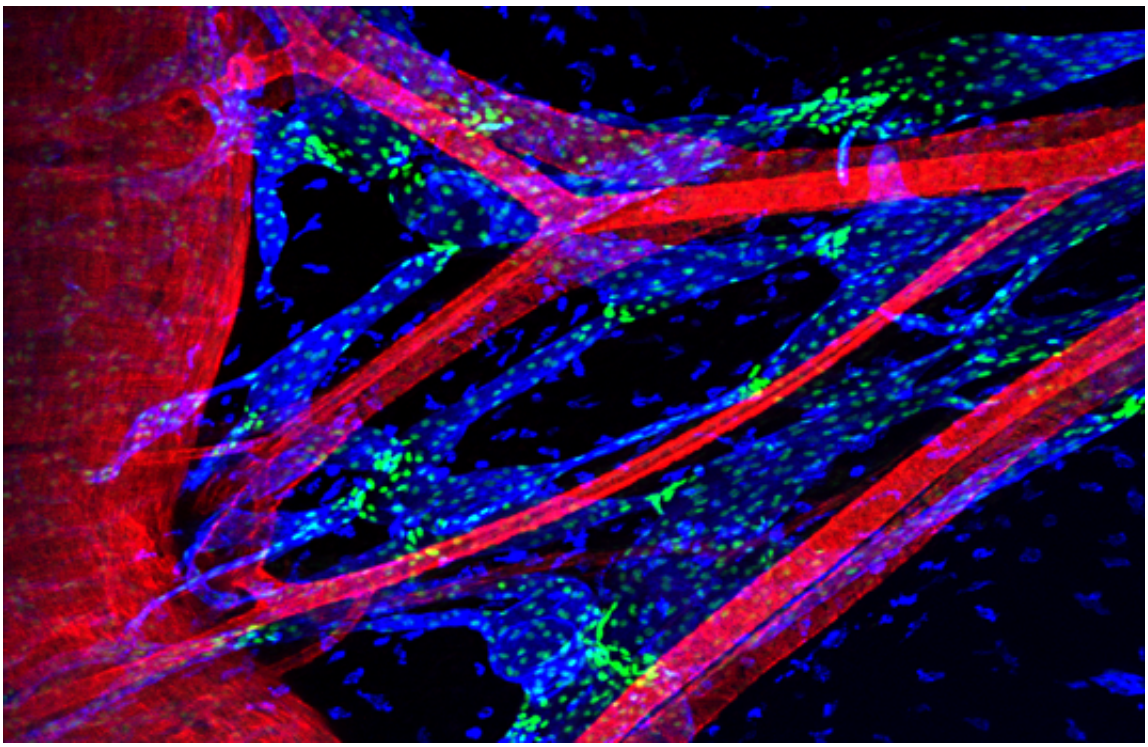

Mesenteric vessels in a mouse embryo: Artery and vein are stained in red, lymphatic vessels in blue. The lymphatic valves are shown in green.

The lymphatic vasculature is vital for the function of the immune system, but its development is poorly understood in comparison to that of the blood vasculature from which it arises. The vessels that make up

the lymphatic network permeate the body and transport the lymph, the fluid into which lymphocytes circulate. Defects in the function of the lymphatic vessels and lymphatic valves are associated with serious diseases. Now a research team based in LMU's Department of Pharmacy has elucidated an important step in the regulation of lymph system development. The study, which was led by Dr. Johanna Liebl and Professor Stefan Zahler at the Institute for Pharmaceutical Biology headed by Prof. Angelika Vollmar, demonstrates that the protein Cdk5 plays an essential role for the morphogenesis of lymphatic vessels.

The results are reported online in the journal *Nature Communications*.

Lymphatic vessels originate from specific veins, but little is known about the molecular mechanisms that direct the patterning of the network. Among the few specific proteins that have been implicated in the process is the transcription factor Foxc2. Transcription factors are responsible for controlling the ordered, tissue-specific readout of the genetic information stored in the DNA, which encodes the amino-acid sequences – and hence the structure and function – of the proteins required in each of the many cell types in the body. Foxc2 is known to be crucial for the formation of various vital organs during embryonic development, and plays a central role in the differentiation of lymphatic valves. Defects in the Foxc2 protein, caused by mutations in the corresponding gene, are known to cause lymphedema-distichiasis syndrome, a rare congenital condition characterized by severe lymphedema. In addition, Foxc2 has been linked to the formation of metastases by certain types of malignant tumors. "But nothing was previously known about how the function of Foxc2 is regulated," says Johanna Liebl.

A critical interaction

Many transcription factors, however, have been shown to be regulated by

phosphorylation –the attachment of phosphate groups at specific positions in a target protein – by enzymes called protein kinases. One such enzyme, Cdk5, has been extensively investigated in relation to its role in the development of the central nervous system. Liebl and her colleagues now analyzed the function of Cdk5 in the endothelial cells that line the walls of both blood vessels and lymphatic vessels. To do so, they used a mouse model system in which the gene for Cdk5 could be inactivated specifically in the endothelium.

"We found that genetic knockout of Cdk5 in the endothelium of the mouse leads to pronounced lymphedema and to embryonic and early post-natal lethality," Liebl says. In the absence of Cdk5, maturation of newly formed lymphatic vessels and the development of lymph valves is profoundly disrupted. As a consequence of the lack of functional valves, the so-called lymph sacs, which are the first lymph vessels to develop, are not properly separated from the blood vasculature. This in turn leads to lymph edemas, as blood leaks into, and accumulates in the lymphatic vessels.

"We went on to show that Cdk5 phosphorylates Foxc2, and that this modification is essential for its function as a transcription factor. Thus our work demonstrates for the first time that an interaction between Cdk5 and Foxc2 is critical for the correct development of lymphatic vessels," Liebl explains. Moreover, these findings do more than provide novel insights into the molecular mechanisms that underlie the development of the lymphatic vasculature. "Since Cdk5 represents a promising target for the development of new drugs, we hope that the results of our study will lead to new therapies for the treatment of diseases associated with defects in the lymphatic vasculature," Liebl adds.

More information: "Cdk5 controls lymphatic vessel development and function by phosphorylation of Foxc2" *Nature Communications* 6,

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