New insights in how blood vessels increase their size

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A new study from the group of Holger Gerhardt (VIB/KU Leuven/Cancer Research UK/ MDC/BIH Berlin) in collaboration with Katie Bentley's Lab (Cancer Research UK/BIDMC-Harvard Medical School) addresses a long standing question in the wider field of developmental biology and tissue patterning in general, and in the vascular biology field in particular: 'What are the fundamental mechanisms controlling size and shape of tubular organ systems'. Whereas the most obvious way to grow a tube in size would be to add more building blocks (by proliferating cells) to enlarge its circumference, or to increase the size of the building blocks (the cells, hypertrophy), an alternative way would be to rearrange existing building blocks. Benedetta Ubezio, Raquel Blanco and colleagues under the direction of Holger Gerhardt and Katie Bentley now show that cell rearrangement is the way blood vessels switch from making new branches to increasing the size of a branch. The researchers also found that this switch is triggered by synchronization of cells under the influence of increasing levels of the growth factor VEGFA.

Holger Gerhardt (VIB/KU Leuven/Cancer Research UK/ MDC/BIH Berlin): "This mechanism also drives vessel enlargement in disease models including a model for diabetic retinopathy and cancer. These insights might lead to new ways of looking at VEGF treatments for cancer."

Cells move in groups

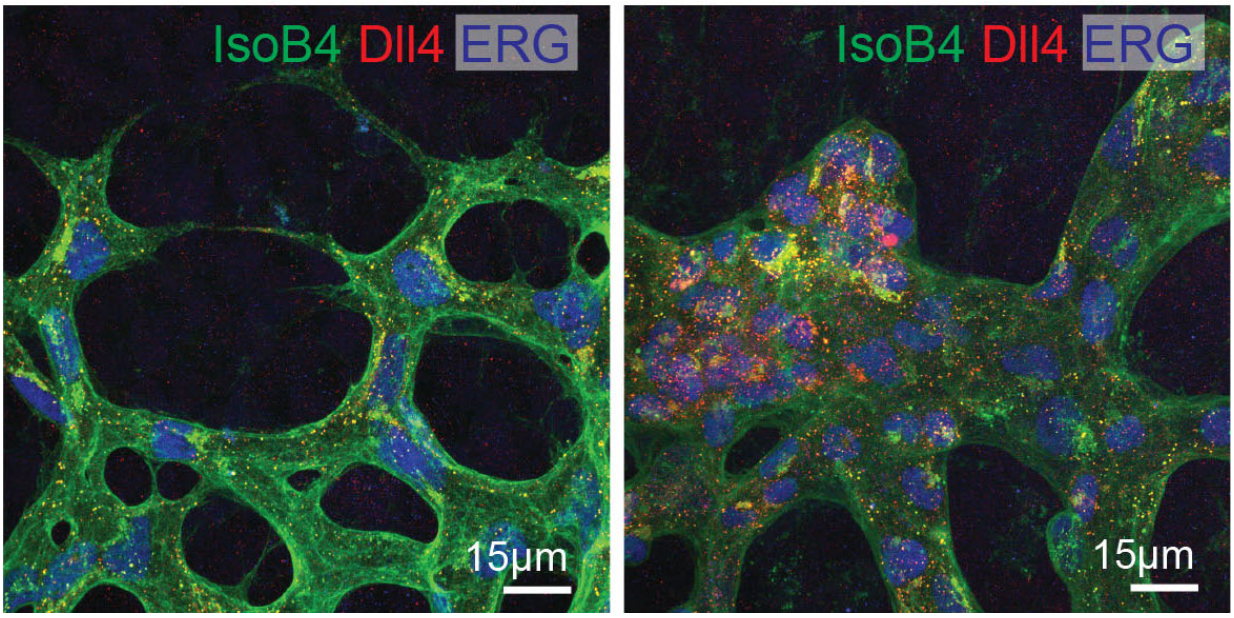
The mechanism of synchronization is Notch signaling, a cell-cell communication pathway that is used by most cells in all organisms, but in many different ways. The present work is the first to directly show that endothelial cells undergo dynamic fluctuations in the activity of this pathway within single cells, and that cell-cell signaling, the strength of which increases when there is more VEGFA, leads to synchronization of the "phase" of these fluctuations.

Katie Bentley (Cancer Research UK/BIDMC-Harvard Medical School): "As a consequence, the cells behave as a collective, moving in the same direction together, rather than moving in different directions as individuals. Branching requires neighboring cells to be "out of phase" in their Notch activity, whereas diameter increase is achieved by clustered movement 'in phase'."

A finding with broad implications

On the one hand, this means that when trying to understand mechanisms of tissue patterning, we need to understand not only what signals (genes, molecules etc) are present and used between the cells and their environment, but also how they change in amplitude and duration over time, within individual cells and within collectives of cells.

Katie Bentley (Cancer Research UK/BIDMC-Harvard Medical School): "This study emphasizes the need to search for mechanisms and tissue factors that alter the dynamics of core signaling pathways, and thus opens our eyes to new possible "temporal dynamic" modifiers in disease that can either explain pathology or open up new targets for unexpected treatments. "



On the left the new blood vessels are in branching mode; on the right they are increasing in diameter under the influence of the VEGFA hormone. The cell membrane is colored green, the cell nuclei blue, and the oscillating signal molecules red. Credit: eLife

Holger Gerhardt (VIB/KU Leuven/Cancer Research UK/ MDC/BIH Berlin): "From a wider methodology perspective it is clear that this work would not have been possible without implementing predictive computational modeling to guide and refine the experimental approaches and analysis. The iteration between modeling and experimentation is what gave rise to the first synchronization hypothesis and ultimately allowed us to ask the right question and perform the key experiments. As such, it is another example of the power of collaborative interdisciplinary work that will increasingly become necessary to tackle the most challenging questions in science and society."

Katie Bentley: "Untangling the complex dynamics of biological pathways in health or disease is almost impossible to do in ones' head. Computer

Simulations are an easy way to watch the process unfold in front of you to identify new lines of enquiry. It was electrifying to see those first unexpected synchronized fluctations and expanding vessels in our high VEGF simulations, and after the dedication of a team of biologists over many years, performing, sometimes gruelingly long experiments! We can see that putting model predictions to the test, a feat still rare even with growing computational studies, can truly lead biology in new directions."

Holger Gerhardt (VIB/KU Leuven/Cancer Research UK/ MDC/BIH Berlin): "The ability to study fluctuations of gene expression live is fascinating, but requires complicated gene editing and transgenic approaches. Whilst this worked OK in the case of our embryonic stem cell derived cultures, the fact that dynamic signals don't accumulate made it very hard to actually detect the low level signals in the tissue in vivo. We will need better, brighter reporters, whilst maintaining their ability to degrade rapidly, in order to watch this behavior unfold in real time in living organisms. This is one aspect we are working on now."

Questions for the future

One area of intense research that is related to this finding is the integration of chemical signals or cell-cell communication with physical signals. Blood vessels are under constant strain and stretch, both by blood pressure and blood flow.

Holger Gerhardt (VIB/KU Leuven/Cancer Research UK/ MDC/BIH Berlin): "Although we know that these effects play an important role in changing vessel size in adaptation to changing flow conditions, the mechanisms are poorly understood. We will now need to understand how the VEGFA-Dll4-Notch pathway and dynamic behavior of it integrate or intersect with signals that derive from blood flow and its physical properties."

More information: Benedetta Ubezio et al. Synchronization of endothelial Dll4-Notch dynamics switch blood vessels from branching to expansion, *eLife* (2016). DOI: 10.7554/eLife.12167