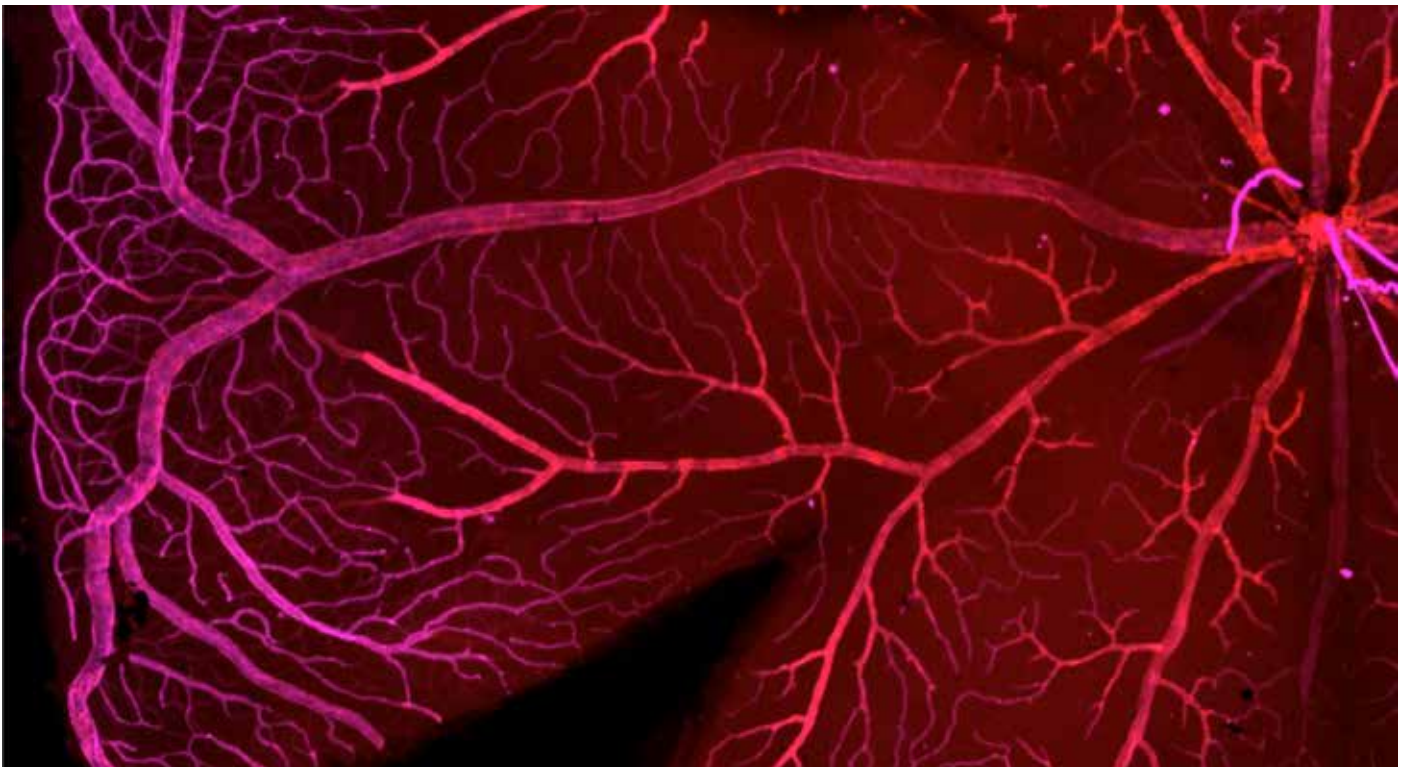


Targeted treatment for venous diseases with vascular system modelling

Lauri Eklund does research on disturbances in the growth and functioning of blood vessels. The aim is, among others, to develop targeted treatments for venous diseases.



Venous diseases mean chronic and progressive vascular changes for reasons that are often unknown. Disturbances in the venous circulation, for example, are common in the retina of the eye, causing impaired vision. Venous malformations, on the other hand, represent rare congenital disturbances in blood vessel formation often caused by somatic mutations in genes that are important for normal venous morphogenesis. Professor Lauri Eklund's research group aims to identify physiological mechanisms which control the differentiation of blood vessels into veins, and cellular and molecular defects may underlie vascular abnormalities.

"The basic mechanisms of capillary formation and differentiation of arteries are known relatively well, whereas there are still a lot of open and interesting bio-

logical questions with the differentiation of veins. In addition, revealing molecular mechanism causing vascular diseases is necessary to the development of targeted therapies"

Based at Biocenter Oulu and at the Faculty of Biochemistry and Molecular Medicine, Eklund does research on the mechanisms controlling the development of veins using genetically modified mice and endothelial cell cultures to model mutations identified in patients and phenomena related to vein formation.

Disturbances in venous circulation in the retina of the eye is a relative common cause of impaired vision. Eklund's research group has recently discovered the first growth factor that is needed for the formation of veins in the retina of a mouse. The growth factor is a protein which stim-

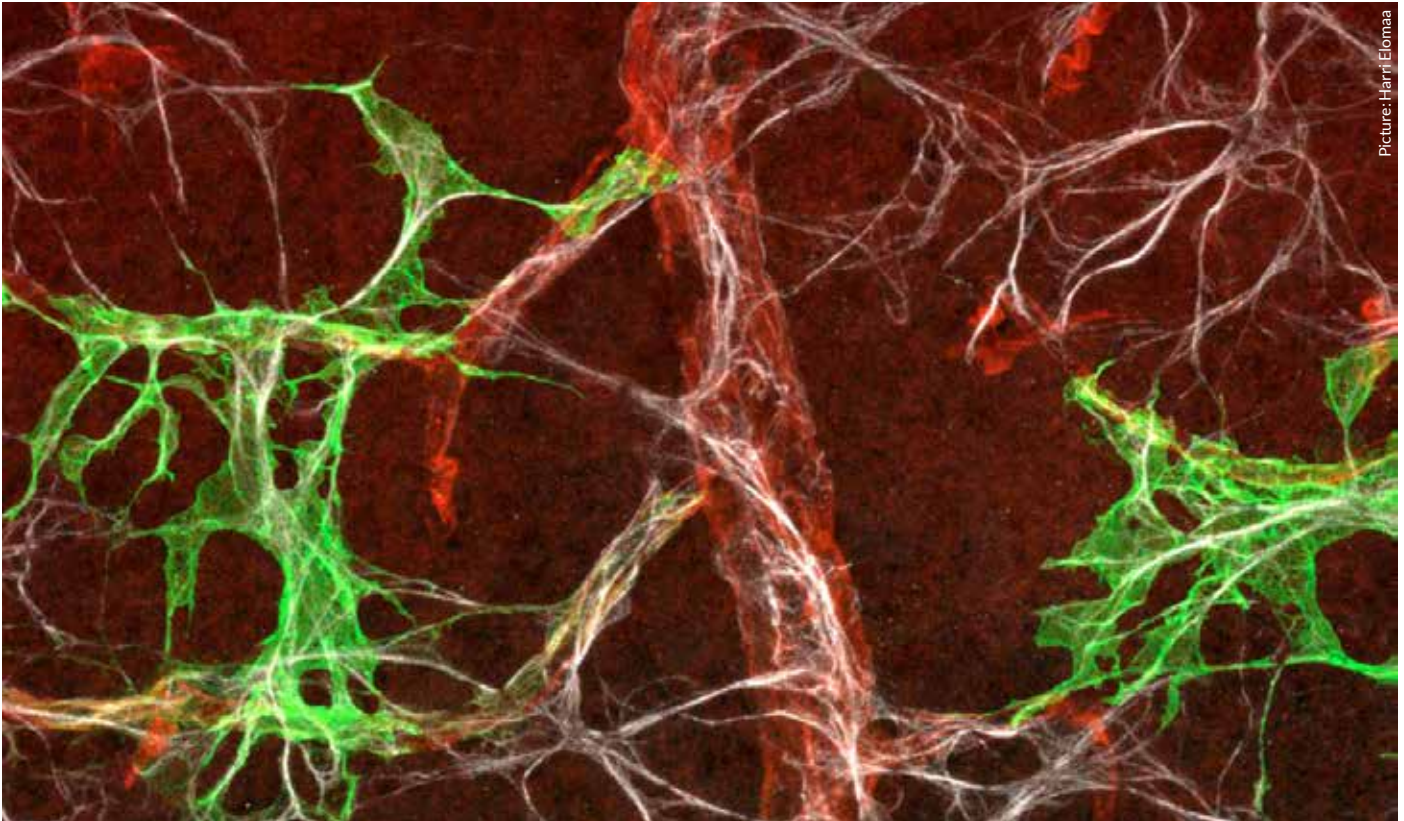
ulates the growth or differentiation of cells. The growth factors also function as signal molecules between cells.

"The importance of the growth factor studied by the group, angiopoietin-4 (Angpt4), has been poorly known until this day. It controls the functioning of TIE2 receptor tyrosine kinase in endothelial cells which cover the inner surface of blood vessels."

TIE2 receptor and disturbances in intracellular signalling

Identification of the TIE2/Angpt-4 cell signalling pathway in normal development of veins is, according to Eklund, a very interesting finding.

"The somatic mutations leading into gain of function of the same TIE2 receptor cause a major proportion of the venous



Picture: Harri Elomaa

The retinal vein, capillaries and glial cells. Angiopoietin (Angpt 1–4) growth factors and their endothelial cell membrane receptor Tie2 are in a key position in terms of blood vessel development, remodelling and permeability regulation.

Angpt4 plays a major role in the creation of veins in the mouse retina. Biocenter Oulu provides advanced microscopic imaging that can analyse cellular and molecular events and thereby identify the mechanisms in normal development and causes of illnesses. (Figure: Laser scanning confocal microscopy of genetically modified, GFP expressing fate mapping mouse.

malformations which occur in humans.” Another prevalently mutated gene is PIK3CA, which corresponds to the activating subunit of PI3K lipid kinase.

Both TIE2 and PI3K are kinase enzymes which control the functioning of their target proteins by phosphorylation. The cells use this mechanism in their normal cell signalling.

“In collaboration with **Miikka Vikkula’s** group (the de Duve Institute, Brussels, Belgium) we have demonstrated that the cell membrane receptor TIE2 and the downstream signal transducer PI3K are located in the same signalling pathway causing venous malformations. The switch-on in TIE2 or PI3K active kinase domains triggered by a mutation will cause the same kind of abnormalities in endothelial cells and vascular structures. The impact of mutations is that they activate cells, causing a false, growth factor in-

dependent and uncontrolled locking-on of the TIE2/ PI3K signalling activity.”

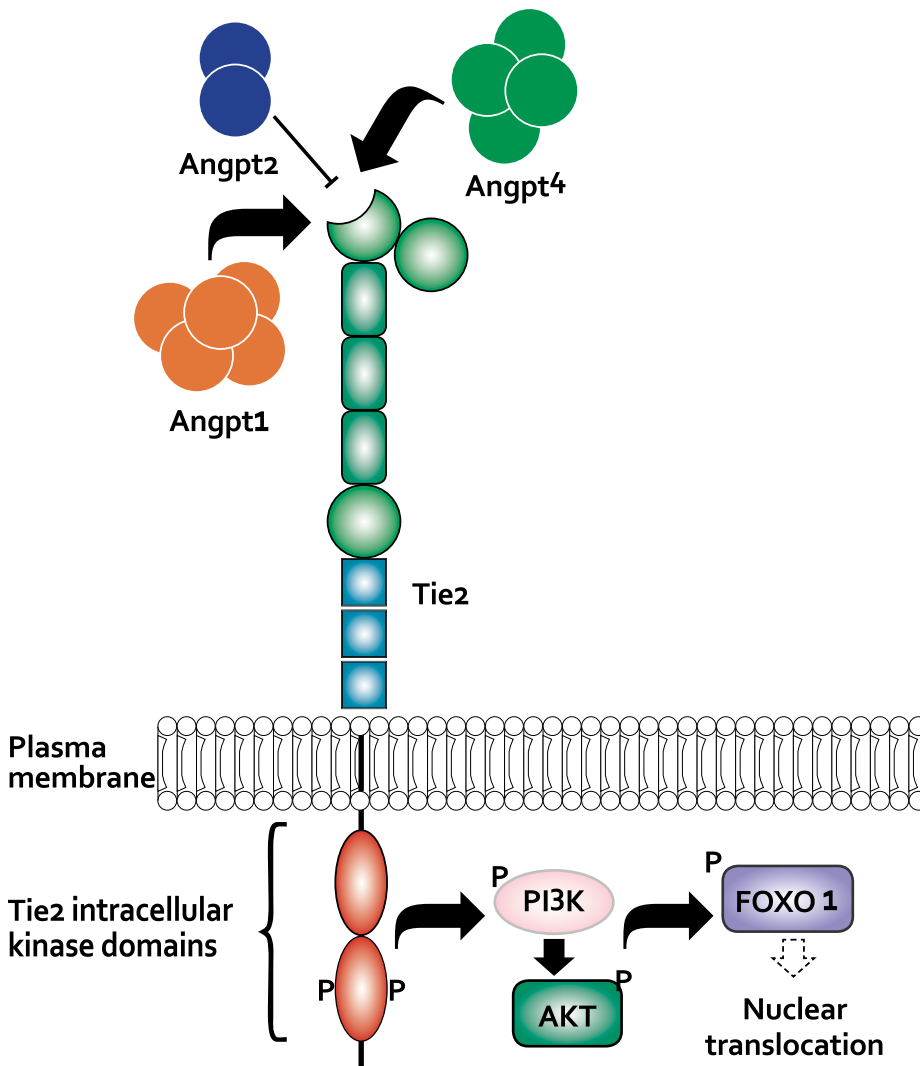
According to Eklund, these observations together refer to the Angpt- 4/TIE2/ PI3K signal route playing a specific role in vein formation in development and disease. Venous malformations also have a surprising connection with the cancer. In DNA certain nucleotide sequences are known as “hot spots”, where mutations concentrate in some diseases.

“The same hot spot mutations in the activating subunit gene (PIK3CA) of lipid kinase PI3K cause not only a significant proportion of venous malformations, but several cancers as well. It is very interesting, yet at the same time poorly understood, how the same PIK3CA mutations cause various malignant cancers in epithelial cells and “only” excessive growth of vein-like channels when occurring in the endothelial cells.”

“Some of the developmental defects in blood vessels are mostly cosmetic “birthmarks”, which can also heal on their own. According to Eklund, one example of problems of this kind are the congenital hemangiomas, such as strawberry naevuses in which the originally excessive endothelial cell division and capillary formation reduces as the child grows older. Hemangiomas are, in fact, the most common benign self-healing tumours, which are typically identified in children at the age of a few weeks. In many of the patients, they have spontaneously regressed by the age of five years.

“What factors cause the cessation of excessive proliferation of endothelial cells is an interesting, yet not well understood phenomenon,” Eklund says.

Although some of the diseases under research are rare, they can help us understand the disease mechanisms and uncover



Angpt/Tie2 cell signalling pathway. Angpt1 is the growth factor that activates the Tie2 receptor. PI3K/AKT is a major intracellular signal transducer, stabilising the vasculature by regulating the FOXO1 transcription factor, for example. In vascular endothelial cells, Angpt2 is a growth factor that can antagonize the activation of Tie2 receptor. This can promote formation on new blood vessels and increase permeability. Until now, the physiological importance of Angpt4 growth factor has been poorly understood. Lauri Eklund's research group has used a genetically modified mouse retina model to demonstrate that the Angpt4 is important for venous maturation.

typical control mechanisms related to the growth and functioning of blood vessels.

"Even though vascular developmental defects have mainly to do with rare diseases, they can also occur in veins, capillaries or lymphatic vasculature. As venous malformations, most of them may likewise originate from gene mutations, yet through other cell signalling routes. Considering the various types of vascular

development defects, they are relatively common together."

After the defective molecule has been identified, it is possible to develop targeted molecular treatments.

"These include small synthetic molecules that are aimed to only target abnormally functioning molecule and do not cause any disturbance to the rest of the events in the body. The efficacy of the

drug improves, and there are fewer side effects."

According to Eklund, identifying the disease mechanisms may also lead to repurposing the drugs for illnesses which they were not originally designed for. One example is, for example, the PI3CA inhibitor Alpelisib.

"Although originally developed as cancer medication, it is also efficient in preventing the development of venous malformations in cell and animal models. The genetic change causing the disease is the same in both cases, but the mutation takes place in different cell types."

Since pharmaceutical companies may not have interest in developing drugs for small patient groups, this kind of 'repurposing' is, as Eklund sees it, often the only opportunity for patients suffering from rare diseases to receive targeted medicinal treatment.

In rare diseases, identification of the mechanisms causing the abnormalities may also uncover yet unknown cell signalling pathways that are involved also in normal growth.

"In fact, many cell signalling pathways and mechanisms which are dysregulated in vascular diseases, are also needed with normal vascular development. One of these is the angiopoietin/TIE2/PI3K pathway that has been under investigation in our research."

Normal vascular development and tumor angiogenesis

The earliest vascular structures in embryos originate from cells known as angioblasts which can differentiate into endothelial cells to form blood vessel. During embryonic development and growth, new vessels are formed from existing ones in a process known as sprouting angiogenesis. In the following maturation phase, the vascular system differentiates into a hierarchic network of functional arteries, veins and capillaries, which is needed for keeping tissues homeostasis. In healthy adults angiogenesis is needed, for example, in recovering from injuries and also occurs for example, in conjunction with menstruation. New formation and changes in the structures and functioning of blood vessels are also related to many diseases. For example, solid tumours are dependent

on blood vessels which feed the tumour with oxygen and nutrients. Blood vessels which have undergone structural changes may accelerate the transition of tumor cells forming metastases into other tissues and so contribute to a disease becoming malignant.

“The neoangiogenesis of a tumour means growth of blood vessels in a solid tumour from the tissue surrounding it. Interestingly, same growth factors that are needed in development also play a role in adverse neoangiogenesis. This kind of growth factors are interesting targets for the generation of pharmaceutical agents. We have investigated one of them, angiotensin-2, which can affect the vascular structures in many ways.”

The extracellular matrix (ECM) is a three-dimensional network of macromolecules between the cells and also supporting the vascular system. Perivascular ECM consists of a specialized, thin layer of macromolecules called basement membrane and in larger vessels fibrillar collagen matrix. Collagens are the largest group of proteins in the extracellular matrix and in the human body as a whole.

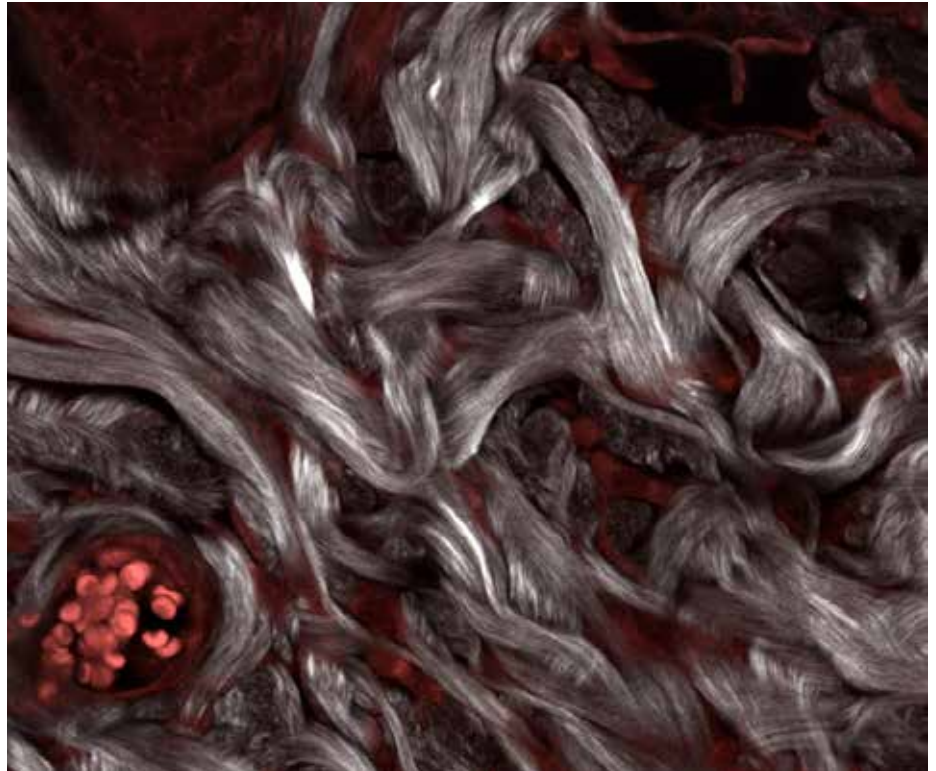
Oulu has a long tradition in ECM and collagen research, and one of Eklund’s aims is to investigate how the endothelial cells and the vascular smooth muscle cells interact with the ECM. Better understanding of the role of the perivascular ECM may help to explain developmental anomalies as well as defects of the vascular system in cancers.

Investigation of disease mechanisms in endothelial cell culture models

The endothelium is an inner single-layer of the blood vessel and also covering inner surface in the heart (endocardium) and lymphatic vessels. Endothelial cells isolated from the organs can be utilised in in vitro studies on culture dishes in laboratories. This enables various tests which cannot be done in living animals or humans,

“The models allow us, for example, to study the effect of growth factors on cells: does the vitality of the cells improve? Do they begin to differentiate or form vascular structures?”

The cell culture models are also used to study mutations found patients. For ex-



Mouse skin collagen and red blood cells. Collagen, which has a strand-like structure, is the commonest protein in connective tissue. Collagen is found in bones, cartilage, tendons, skin for example and supports veins and arteries.

Around 40% of the body’s entire mass of proteins is made of collagen. Fibrillar collagen matrix. In vasculature, there is also a thin, sheet-like layer of specialized connective tissue, called a basement membrane, around the endothelial cells on the interior wall of blood vessels. The basement membrane binds growth factors, gives structural support and regulates permeability (Figure: Multiphoton microscopy of mouse skin, label-free second harmonic generation, Veli-Pekka Ronkainen & Ritva Heljasvaara).

ample, they can be investigated to see how cells change when they express a mutated form of a gene. In 3D cultures, endothelial cells can be used to grow structures which resemble blood vessels.

According to Eklund, the opportunities for manipulations and research methods in cell-culture models are almost limitless.

“To better understand the normal development and diseases, often very complex entities can be divided into smaller or single events. Another benefit of cell culture models is that cells of human origin can be used. In some cases, the treatments developed using non-human systems do not have the same effect in the patients. In other words, the results gained e.g. with mice are not necessarily transferred to humans as such. Cell culture models also reduce the need for

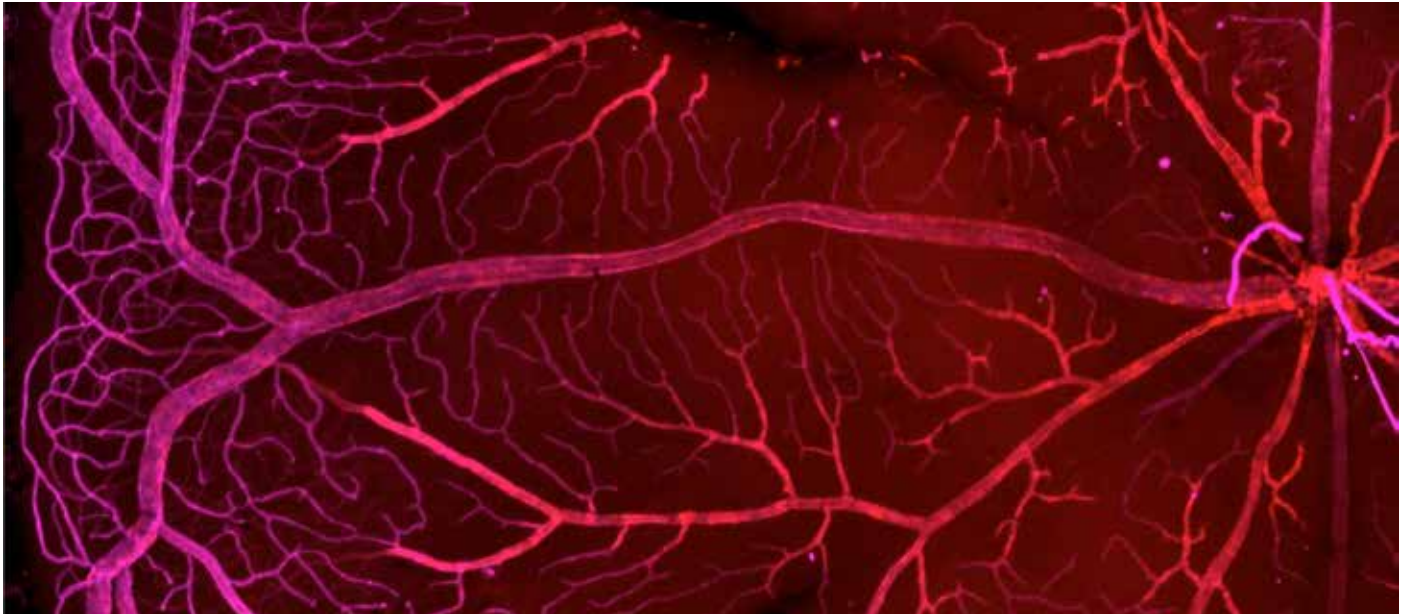
animal testing, and they are not subject to similar research-ethical considerations. For example, endothelial cells of human veins are derived from umbilical cords of volunteer donors after childbirth.

In Eklund’s research group, the formation of vascular structures are also modelled in a 3D environment.

“Even though they imitate tissues better than two-dimensional cultures on plastic dishes, they still fail to have the natural flow of blood vessels”.

In addition to European partners, Lauri Eklund’s research group is collaborating with the FICAM centre at the University of Tampere and an Oulu-based start-up company Finn Advance in a project financed by the EU.

One purpose is to develop “microfluidic” flow channels coated with human



The retinal vein (in the middle) in a mouse. Venous disorders are common blood vessel problems. Venous insufficiency in the lower extremities causes varicose veins. In the eye venous occlusions impairs the vision due to retinal damage. Venous malformations are rare, congenital vascular development disorders caused by somatic mutations. New research findings are being used to understand normal venous development, disease modelling and for seeking medical treatments for vascular diseases.

(Figure: Laser scanning confocal microscopy of immunostained retina flat-mount, Minna Kihlström).

cells and perivascular ECM designed to correspond to normal blood vessels or abnormal structures found in vascular anomalies. The aim is to use the devices to study what the changes in blood flow cause in the vascular system and especially in their malformations, where the blood flow has undergone significant changes.

In many case animal models are still needed for verifying the results.

“If one uses excessively simplified models, the results may become unreliable. Therefore, animal models are still needed for verifying the findings in a more complex environment, which better corresponds to the human tissue. Currently, the mouse is the best one out of the mammalian models

for this kind of research. What makes the mouse models the best is that many genetic modifications are feasible to generate, including the expression of the same gene mutation which causes diseases in humans.”

The research is financed by the Academy of Finland and the EU H2020-MSCA-ITN programme.

Ari Turunen

FOR MORE INFORMATION:

Biocenter Oulu

Biocenter Oulu is part of Biocenter Finland, which coordinates infrastructural activities for major national research. It is also a member of various European research infrastructures. These are Infrafrontier (transgenic mice), Euro-BioImaging (biological imaging) and Instruct (protein structure research).
<https://www.oulu.fi/biocenter/>

CSC – IT Center for Science

is a non-profit, state-owned company administered by the Ministry of Education and Culture. CSC maintains and develops the state-owned, centralised IT infrastructure.
<http://www.csc.fi>
<https://research.csc.fi/cloud-computing>

ELIXIR

builds infrastructure in support of the biological sector. It brings together the leading organisations of 21 European countries and the EMBL European Molecular Biology Laboratory to form a common infrastructure for biological information. CSC – IT Center for Science is the Finnish centre within this infrastructure.
<http://www.elixir-finland.org>
<http://www.elixir-europe.org>

ELIXIR FINLAND

Tel. +358 9 457 2821s • e-mail: servicedesk@csc.fi
www.elixir-europe.org/about-us/who-we-are/nodes/finland

www.elixir-finland.org

ELIXIR HEAD OFFICE

EMBL-European Bioinformatics Institute
www.elixir-europe.org