New actors identified in atherosclerosis

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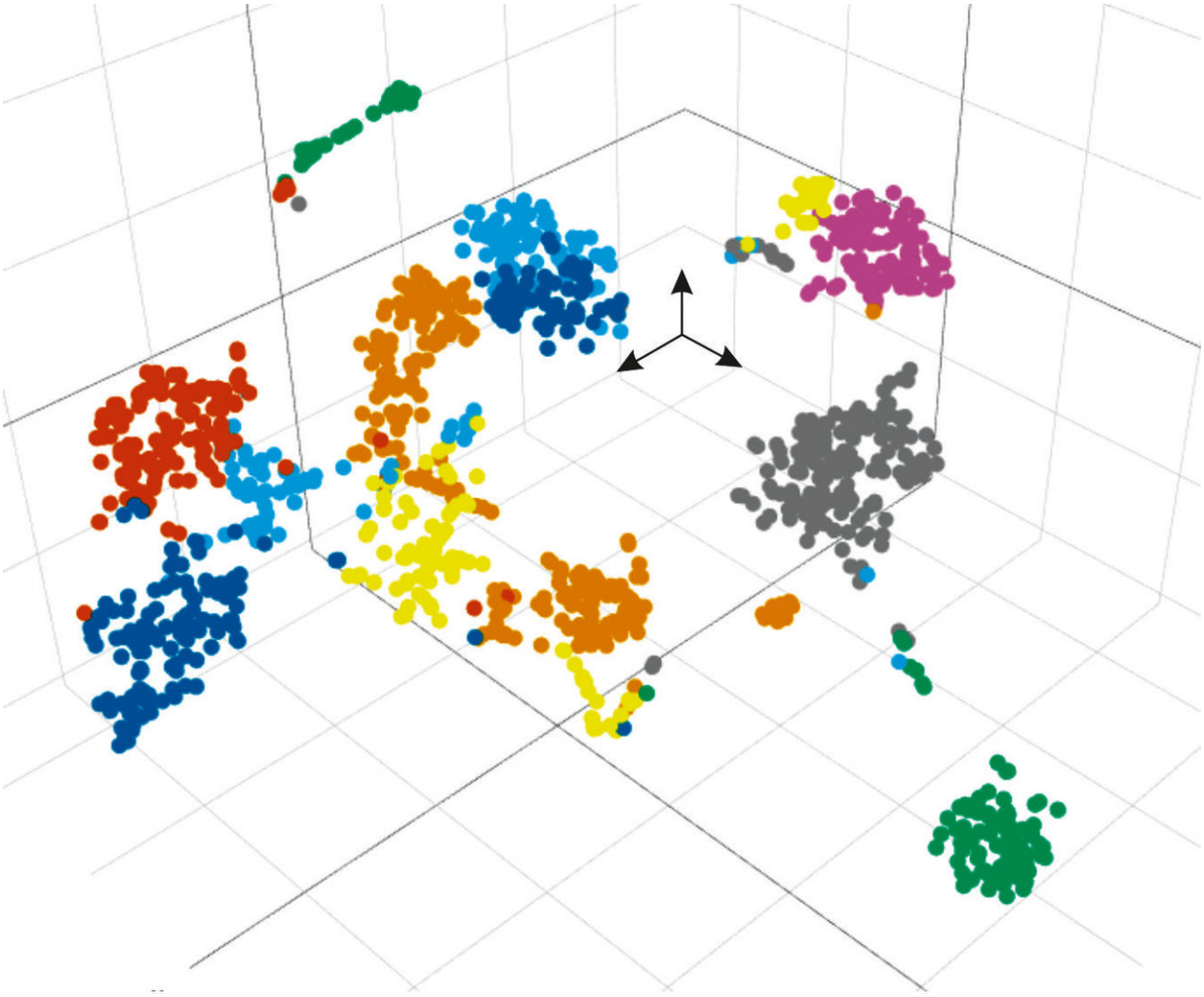

Illustration of the gene expression fingerprint of each single cell in a three-dimensional projection after bioinformatics analysis to identify the cellular phenotype. Credit: Cochain/Vafadarnejad/Saliba/Zernecke

Stroke and heart attack are the leading cause of death in the Western world. Würzburg scientists have used a special technique to get a clearer picture of the cells involved and their activity.

Atherosclerosis is the most common cause of death and disease in the Western world. In Germany, about 300,000 people each year suffer a heart attack and some 270,000 a stroke as a result of the condition. Atherosclerosis is estimated to be responsible for a little more than half of all deaths in these countries.

Searching for the disease triggers, scientists from the University of Würzburg and the Würzburg University Hospital have now made a step forward: For the first time, they closely examined the immune cell populations in the affected vessels which play a significant role in the pathogenesis. They present their results in the current issue of the journal *Circulation Research*.

"We used single-cell RNA sequencing to identify three different macrophage populations that can influence the development of atherosclerosis in different ways. This includes a macrophage population that has not been described previously," Clement Cochain sums up the central result of the recently published study. These populations were encountered in different disease stages as well as in different disease models.

The study was conducted by Professor Alma Zernecke-Madsen and her team at the Department of Experimental Biomedicine II at the Würzburg University Hospital in close cooperation with Dr Emmanuel Saliba and his team at the Helmholtz Institute for RNA-based Infection Research (HIRI) established at University of Würzburg in 2017, a branch of the Helmholtz Centre for Infection Research (HZI).

Plaques with fatal consequences

The risk factors for atherosclerosis are well known and include a high fat diet, lack of physical activity and smoking. But there are still a lot unresolved questions about what happens exactly inside the affected blood vessels. The disease often begins with tiny damages of the interior vessel wall that lead to chronic inflammation.

Subsequently, fats and other constituents of the blood deposit on the vessel walls, and immune cells from the blood migrate to the damaged area where they produce signalling substances to summon more immune cells. This leads to the formation of the so-called atherosclerotic plaques which increasingly clog the blood vessels. If they become detached from the vessel wall, the plaques can travel with the blood stream to other places in the body, e.g. the leg or head, and block circulation.

An exact image of the cells involved

"It has already been suspected that different macrophage subsets are at work in atherosclerotic vessels where they assume different tasks," Clement Cochain and Alma Zernecke-Madsen explain. In the past, however, scientists always failed to identify them because the suitable markers were not available. The use of single-cell RNA sequencing has now allowed the Würzburg team to be more successful.

This technique isolates the RNA molecules in single cells and analyses them using high-frequency sequencing. "The data delivered by this method provide information on the gene expression of each individual cell and give insight into their very special functions," Dr Emmanuel Saliba explains.

Aorta cell samples analysed

The scientists took cell samples from a healthy aorta and an

atherosclerotic aorta and sequenced them. They identified two types of macrophages only in the diseased vessels, namely monocytes and the so-called dendritic cells that derive from monocytes. In healthy vessels, they found local macrophages and several other immune cells.

As part of the immune system, monocytes circulate in the blood stream before migrating into the tissue to differentiate further to specific tissue macrophages. They are also part of the cell-mediated immune response and reside in the human body for several months.

According to the scientists, the new data provide a hitherto unknown picture of the immune cells in atherosclerotic plaques during atherosclerosis. "These findings open up new possibilities to study these cell populations and their respective functions during atherosclerosis in greater detail now," Alma Zernecke-Madsen says.

More information: Clément Cochain et al. Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis, *Circulation Research* (2018). DOI: 10.1161/CIRCRESAHA.117.312509

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