Could you explain the evidence that has linked macrophage activation with atherosclerosis – and its acute complications?

Macrophage activation contributes to the pathogenesis of various conditions, including atherosclerotic vascular diseases. Some complications of atherosclerosis cause acute myocardial infarction, which is a leading cause of death in developed countries such as the US. Metabolic characteristics, including elevated cholesterol level, can promote macrophage activation. Preclinical evidence, including our own, demonstrated that cholesterol lowering can reduce macrophage activation in experimental atherosclerosis. Moreover, clinical evidence has also established that cholesterol lowering reduces vascular inflammation. However, even after intense research efforts in this area, many patients still suffer from heart attacks. In view of this, new medical solutions are needed to tackle residual risks.

Your latest project focuses on Delta-like ligand 4 (DLL4) and Notch signalling in macrophage activation; what are its chief objectives?

In view of the serious health burden imposed by complications associated with atherosclerosis, the researchers in my laboratory have explored new mechanisms for macrophage activation, which extend beyond conventional modifiable risk factors. About a decade ago, when I began studying key signalling mechanisms, I noticed that the role of Notch signalling in macrophage biology was unknown. This motivated me to begin a project with my summer student, Eric Fung, who later became a research fellow in my lab. In our project, we found that upon inflammatory stimuli human macrophages could express DLL4 – and this marked the beginning of our extensive research into DLL4.

How is your team dissecting the complex and intertwined mechanisms of macrophage activation?

We found that if DLL4 binds to macrophages it triggers Notch signalling that induces pro-inflammatory genes and pathways such as INOS and NF-κB. DLL4 also promotes the expression of DLL4 itself. In a key article that we published in 2007, we thus proposed that DLL4 accelerates a positive feedback loop of pro-inflammatory activation of macrophages. We then tested this working hypothesis in vivo using several mouse models. Importantly, we discovered that DLL4 blockade attenuated multiple features of vascular and metabolic diseases, including arterial plaque development, macrophage activation, calcification, excessive body weight gain, insulin resistance and fatty liver. We thus concluded that DLL4-Notch signalling contributes to the shared mechanisms for such cardio-metabolic disorders, which have become global health threats. Furthermore, we have also established several mouse strains in which we selectively manipulated Notch components in macrophages to examine the role of each molecule in macrophage activation and vascular disease.

To what extent does a collaborative and multidisciplinary approach benefit your research?

Medical sciences have progressed so rapidly and have become so complex that a collaborative and multidisciplinary approach has become necessary to make advances. Personally, I have very much enjoyed cooperating with many investigators with different backgrounds and skillsets. In addition to multidisciplinary collaborations...
Combating cardiometabolic conditions

Researchers at Brigham and Women’s Hospital in Boston, USA, are forging insights into the molecular mechanisms behind atherosclerosis and their contribution to the pathogenesis of severe cardiovascular diseases.

Where do you foresee the next big advances in your field, and in the health sector in general?

There are many potential breakthroughs. However, I am particularly excited about the prospect of establishing more individualised disease mechanisms as well as exploring common mechanisms. Personalised therapies have already come under scrutiny – and the hope is that this trend will be further accelerated in the future. My lab uses multiscale modelling involving mouse and human cells, primary cells from these species, mouse models and human tissue samples, which help us establish unambiguous and clinically translatable evidence. If you look at gene expression profiling of human primary macrophages derived from circulating monocytes, there are huge donor variations. This is often puzzling and difficult to manage experimentally, but it could prove to be an important feature that underpins the development of efficient personalised therapies. Every single human is unique and by incorporating various new methods, such as proteomics and systems biology, in investigations of human samples, I believe we could identify new mechanisms that are unique to certain populations.

Atherosclerosis is a chronic condition in which the arteries become clogged up with fatty substances. It typically begins with damage to the endothelium – that is, the thin layer of cells that line the arteries and keep the blood flowing smoothly – with cholesterol crossing the damaged endothelium and entering the artery walls. In turn, white blood cells that attack foreign material – namely, macrophages – stream into the arteries to digest the cholesterol. Over a period of many years, the mass of cholesterol and blood cells accumulates to form plaque on the artery walls, causing the arteries to become harder and thicker. Eventually, this leads to restricted blood flow that can damage the organs and lead to serious cardiovascular conditions including peripheral arterial disease, coronary heart disease, strokes and heart attacks.

Worryingly, atherosclerosis and the diseases it triggers represent a significant threat to global health. Indeed, heart attacks are the most common cause of death in the US and other developed countries – and the worldwide prevalence of ischemic heart disease increased by 29 per cent between 1990 and 2010. Additionally, related metabolic disorders such as obesity, Type 2 diabetes and fatty liver are also major public health and clinical problems in the western world.

However, in spite of the widespread prominence of cardiometabolic disorders, the mechanisms that underpin arterial inflammation are still not fully understood. In response to this knowledge gap, Dr Masanori Aikawa – Associate Professor of Medicine at Harvard Medical School and Director of the Center for Interdisciplinary Cardiovascular Sciences at Brigham and Women’s Hospital (BWH) – is leading a study that uses mouse models to explore the molecular signalling and pathways that contribute to atherosclerosis. With recent studies having linked the activation of macrophages to atherosclerosis, obesity, insulin resistance and fatty liver, Aikawa’s laboratory is researching the mechanisms that underpin this activity. Together, the scientists are using a combination of in vitro and in vivo approaches, spanning macrophage-selective knockout, transgenic mouse models, biotherapeutics, and molecular imaging of atherosclerotic plaque macrophages. The goal is for these investigations to lead to the discovery of new therapeutic targets for vascular diseases, as well as contribute to the development of preventative cardiovascular treatments.

Mapping the mechanisms

Aikawa’s study is fundamentally multidisciplinary. His team is exploring whether Delta-like ligand 4 (DLL4)-triggered signalling contributes to the
functions of the Notch pathway

INTERNATIONAL CARDIOLOGY, CARDIOVASCULAR RESEARCH, VASCULAR BIOLOGY

board member for several major medical articles and he has served as a reviewer for Aikawa’s studies have been documented in over and the pro-inflammatory role of dyslipidemia. and metabolic disorders, macrophage biology, and cardiometabolic diseases

KEY COLLABORATORS

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INTELLIGENCE

DLL4 IN MACROPHAGE ACTIVATION

OBJECTIVES

• To test the novel hypothesis that DLL4-triggered signalling contributes to the pathogenesis of atherosclerosis
• To examine which Notch receptors (eg. Notch1 vs Notch3) mediate macrophage activation and athrogenesis
• To offer novel mechanisms of macrophage activation and atherosclerosis, and provide proof of concept that the DLL4-Notch3 pathway can be a therapeutic target for atherosclerosis, its complications, and other cardiometabolic diseases

INVESTIGATING DLL4-NOTCH SIGNALLING

As an evolutionarily conserved pathway in multicellular organisms, Notch signalling regulates cell fate during development and maintains homeostasis in adult tissue, as well as playing an important role in immune mechanisms and metabolism. Previous studies conducted by Aikawa and his team have found that the expression of typical M1 markers – such as iNOS and NF-kB – is induced when DLL4 binds to human macrophages. "In particular, our data suggested that DLL4 mediates the positive feedback loop of macrophage activation, which is a typical feature of sustained inflammatory responses in atherosclerotic plaques".

In view of these findings, Aikawa and his team have hypothesised that the DLL4-Notch axis could be a potential therapeutic target for cardiometabolic diseases. For example, a recent in vivo study on the role of DLL4 led by Dr Daiju Fukuda – one of Aikawa’s former postdoctoral fellows – found that DLL4 antibody treatment inhibited the progression of atherosclerotic arterial diseases and metabolic disorders in hyperlipidemic LDL receptor-deficient mice: “Importantly, DLL4 antibodies reduced the M1-dominant microenvironment in metabolic organs, which indicates that this biotherapeutic may treat other inflammatory disorders,” Aikawa adds.

TOWARDS CLINICAL TRANSLATION

Recently, Dr Jun-ichiro Koga of Aikawa’s group analysed the role of DLL4 and Notch receptors in a range of different cardiovascular diseases, with one key study examining the effects of DLL4 biotherapies for vein graft failure. This is a particularly crucial area as vein graft failure is a devastating clinical problem with no medical cure at present – and alarmingly there is a growing number of individuals who need vein graft implantation.

Essentially, a deeper and more nuanced understanding of the role of the Notch pathway in macrophage biology will pave the way for a more complete knowledge of the mechanisms that lead to arterial inflammation. Indeed, the aim is that the basic research conducted in Aikawa’s laboratory will be translated into clinical practice, bringing hope to many patients who suffer with various cardiometabolic diseases.