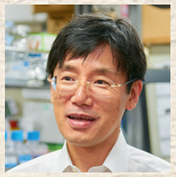
The Discovery of the Molecule's Role in Maintaining Liver Size and Function

THE LIVER IS one of the main detoxifying organs, removing waste and xenobiotics through metabolic conversion and biliary excretion. The waste and xenobiotics come from the gastrointestinal tract via the portal vein, and diffuse into small blood vessels known as hepatic sinusoids. Thus, the liver is constantly exposed to various stresses that can lead to tissue damage. Cellular stress in the liver leads to senescent, transformed, or damaged cells. These cells can impair tissue function or lead to tumorigenesis and therefore need to be eliminated and their loss compensated for by cell proliferation to maintain organ size. However, the molecular mechanisms that act to maintain three dimensional (3D) tissue and organ homeostasis during cellular stress are largely unknown (Fig.1).

The transcription coactivator YAP regulates organ size and cancer formation. Unphosphorylated YAP translocates into the nucleus, interacts with the transcription factor TEAD, and induces target gene expression. Our group isolated a unique medaka fish mutant, hirame (*hir*), which is sensitive to deformation by gravity. *hir* embryos display a markedly flattened body caused by mutation of YAP. We reported that YAP is essential for proper 3D body shape through regulation of cell tension (Nature 2015). In *Drosophila*, the cells with relatively lower fitness levels are eliminated from the tissue by a cell-cell interaction, which is called "cell competition". We found that active YAP-expressing mammalian epithelial (MDCK) cells are eliminated apically when the cells are surrounded by normal MDCK cells (Sci Rep 2016).

Our recent study has shown that YAP regulates the fate of hepatocytes by determining whether they proliferate to boost the organ's bulk or are degraded and removed (Nat Commun 2017). To examine how the Hippo pathway affects the fate of individual hepatocytes, we first established mosaic conditions by using hydrodynamic tail vein injection (HTVi) to introduce active YAP into mouse liver *in vivo*. We discovered that the fate of YAP-expressing hepatocytes changes from proliferation to migration/apoptosis depending on the status (healthy or damaged) of the liver (Fig.2). We also found that the elimination of YAP-activated hepatocytes is regulated by a mechanism distinct from adaptive immunity-dependent senescence surveillance. We found that both CDC42 and Rac which are small Rho family GTP proteins that regulate cytoskeleton organization and cell migration, contribute to YAP-activated hepatocyte elimination. Furthermore, we identified the upstream regulators of CDC42 and Rac in hepatocytes expressing active YAP as Ect2 and Fgd3, which are guanine nucleotide exchange factors (GEF) for CDC42 and Rac. Thus, F-actin formation and YAP activation regulate each other through a feedback mechanism. In summary, YAP acts as a stress sensor that induces the elimination of injured cells to maintain tissue and organ homeostasis. These findings demonstrate the complexity of cell fate determination mechanisms *in vivo*, and highlight a new role for YAP in tissue dynamics.



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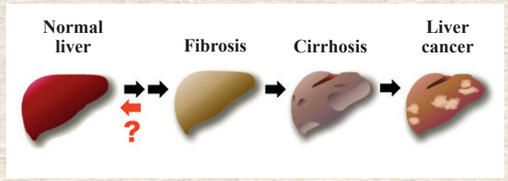


Fig.1: **Liver Homeostasis and Liver Diseases**
The liver is constantly exposed to various stresses that can lead to tissue damage. Cellular stress in the liver leads to fibrosis, cirrhosis and liver cancer.

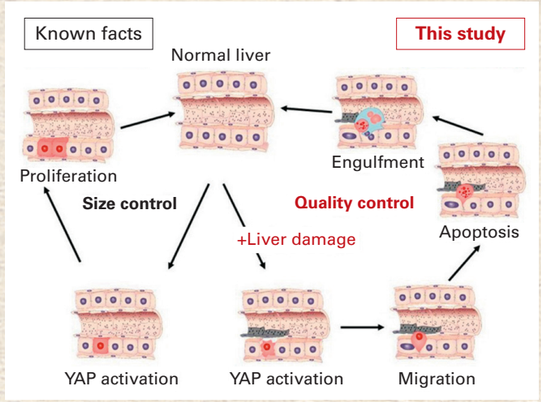


Fig.2: **Schematic Model of the Change in Mouse Hepatocyte Fate**
Active YAP (red cells) regulates liver size through hepatocyte proliferation (left; Previous work). In this study, we showed that active YAP selectively eliminates damaged hepatocytes (right). Hepatocytes expressing activated YAP in the presence of liver injury such as ethanol migrate into sinusoids, undergo apoptosis and are engulfed by Kupffer cells (blue).

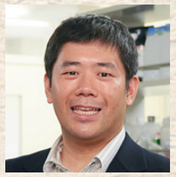
A Lightning-Fast Human Influenza Virus Detector

RESEARCH CENTERED AT Tokyo Medical and Dental University (TMDU) builds a novel biosensor for the influenza virus that is almost 100 times more sensitive than conventional tests, and can distinguish between human and avian strains.

Tokyo, Japan – Researchers have developed a new, rapid biosensor for the early detection of even tiny concentrations of the human influenza A virus. Such early-stage diagnosis is crucial for averting a potential pandemic outbreak, as antiviral medication must be administered in a timely fashion. Conventional tests for detecting the flu virus are often slow and insensitive, and can miss early viral infections. In contrast, the new biosensor measures tiny changes in voltage in an electrically conductive polymer to quickly detect virus amounts almost 100 times smaller than the limit of currently available kits. The work was done at the TMDU, in a collaboration between the Department of Bioelectronics and the Department of Molecular Virology.

Conductive polymers are a class of carbon-based conjugated macromolecules that conduct electricity, but can also be used in biological environments. They are very attractive materials for biosensor applications because researchers can easily attach bioreceptors to the polymers, which allow them to bind with specific targets, such as flu viruses. In this study, poly(3,4-ethylenedioxythiophene) (PEDOT) was modified with a trisaccharide that binds to human flu virus, but not avian flu strains. "Conducting polymers have several advantages over inorganic counterparts," explains corresponding author Yuji Miyahara. "These include the ability to conduct both electrical and ionic carriers, mechanical flexibility, low cytotoxicity, low-cost production by printing, and tunable properties via chemical synthesis or doping."

When a solution containing H1N1, which carries a tiny positive charge on its exterior shell, was added, some of the viruses interact with the polymer (Fig. 1) and increased the voltage. Viral loads are often measured in hemagglutination units (HAU). The new biosensor can detect viral concentrations as small as 0.013 HAU. By comparison, commercially available kits that use immunochromatographic tests only work for concentrations greater than about 1.13 HAU. This represents an almost 100-fold increase in sensitivity. Study coauthor Shoji Yamaoka stressed the clinical applicability of the device. "We developed a conducting polymer-based sensor that can recognize a specific virus, which makes it a good candidate for wearable monitoring and point-of-care testing."



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The article, "Specific Recognition of Human Influenza Virus with PEDOT Bearing Sialic Acid-Terminated Trisaccharides" was published in ACS Applied Materials & Interfaces at DOI: 10.1021/acsami.7b02523

Summary: TMDU researchers built a novel biosensor for the rapid detection of human influenza virus using a bioreceptor-attached conducting polymer. The voltage-sensing detector was almost 100 times more sensitive than conventional tests, and distinguished between human and avian flu strains. The use of this biosensor may provide point-of-care testing and help prevent the outbreak of flu pandemics.

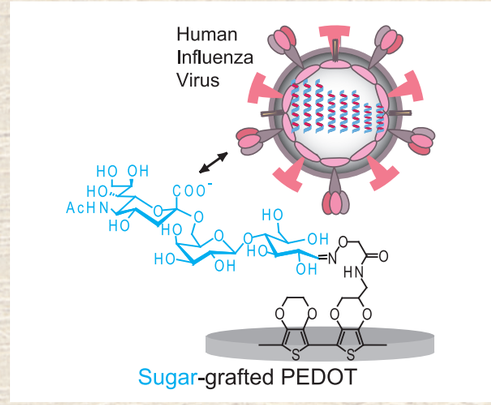

Fig.1: **Human Influenza Virus Recognition by Sugar-modified Conducting Polymers**
A new conducting polymer was developed for detecting specific interaction of trisaccharide with hemagglutinin in the envelope of the human influenza A virus (H1N1) by electrical manners.

Protein Critical to Early Stages of Cellular HIV Infection Identified

TOKYO MEDICAL AND Dental University (TMDU)-led researchers identify a protein critical to the early stages of infection of cells by HIV, offering a potential target for anti-HIV treatment.

Tokyo, Japan – When a virus enters a cell, one of the first steps in the process of infecting that cell is removal of the protein coat that surrounds the virus's genetic material. The virus can then produce DNA from its own genes and insert it into the cell's genome. This allows the virus to hijack the host cell's machinery, forcing the cell to make copies of the virus.

HIV-1 is the most common form of HIV, the virus that causes AIDS. Now, a team led by researchers at TMDU have identified a protein produced by the host cell that is necessary for correct removal of the protein coat of HIV-1. The study was published in *PLOS Pathogens*.

In their search for factors involved in HIV-1 infection, the team interfered with the activity of over 15,000 host cell genes to identify those whose suppression allowed the cells to survive exposure to the virus (Fig.1). This led them to focus on a protein called maternal embryonic leucine-zipper kinase (MELK).

"Depleting cells of MELK reduced HIV-1 infectivity," lead and corresponding author Hiroaki Takeuchi says. "The virus entered the MELK-depleted cell normally, but its protein coat was not removed correctly so it was unable to efficiently produce DNA from its own genetic material. When we restored MELK, the infection process was also restored."

The researchers went on to investigate how MELK interferes with the protein coat removal step of infection. They discovered that MELK alters the coat by attaching a biologically active modification through specific phosphorylation of the capsid at serine-149. This in turn ensures correct removal of the coat (Fig. 2). When the team engineered a mutated version of HIV-1 that was already modified at this location, they found that MELK was no longer needed for coat removal.

"Our results reveal a previously unrecognized mechanism involved in removal of the protein coat of HIV-1 and contribute to our understanding of the early stages of the viral life-cycle," corresponding authors Hiroaki Takeuchi and Shoji Yamaoka say. "Furthermore, our findings suggest that MELK is a potential target for anti-HIV-1 therapy."

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The article, "Phosphorylation of the HIV-1 capsid by MELK triggers uncoating to promote viral cDNA synthesis", was published in *PLOS Pathogens* at DOI: 10.1371/journal.ppat.1006441.

Summary: A TMDU-led research team identified a protein, MELK, required for the HIV-1 to efficiently infect its target cells. MELK, produced by the cell, is necessary for removal of the protein coat around the HIV-1, which is essential for the infection process. The team further revealed that MELK modifies the protein coat through specific phosphorylation of the capsid at serine-149 to promote its removal. These findings offer a potential new target for anti-HIV treatment.

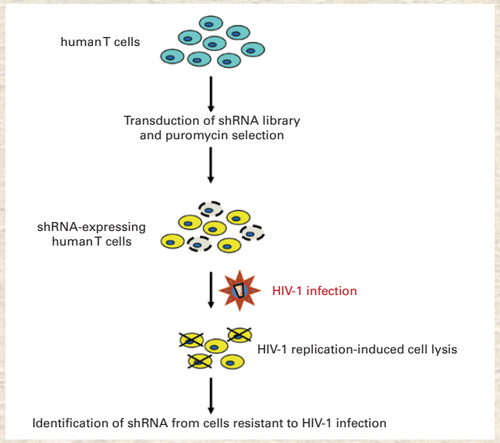

Fig.1: **Schematic Summary of the Genome-wide RNAi Screen to Identify Essential Host Factor(s) for HIV-1 Infection of Human Cells**

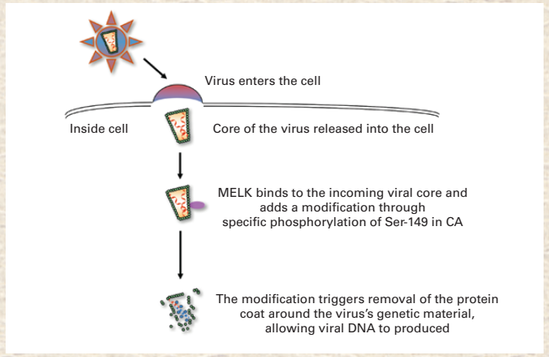

Fig.2: **Modification of the Viral Protein Coat by MELK Regulates Its Removal to Allow Viral DNA Synthesis**
Shortly after HIV-1 entry, MELK produced by the target cell regulates removal of the protein coat (the capsid, CA, which is an important part of the core of the virus) by adding a modification at a specific location. This regulated coat removal promotes optimal production of viral DNA, allowing the infection process to proceed efficiently.

A Novel Synthetic Method for Phosphine Oxides with Three Different Substituents

RESEARCHERS CENTERED AT Tokyo Medical and Dental University (TMDU) have developed a facile method for preparing phosphine oxides bearing three different substituents (Fig.1).

Organophosphorus compounds have a wide application in various fields such as drug discovery and organic materials. Despite the importance of these compounds, synthesis of complex organophosphorus compounds, particularly tertiary phosphine oxides in which three different substituents are bound to the phosphorus atom, was difficult to achieve by conventional methods. This is because these methods often use starting materials with phosphorus-chlorine bonds that are unstable to water and also highly reactive to nucleophilic reagents, making difficult to control the desired sequential substitution reaction. In this context, a more practical method for the synthesis of organophosphorus compounds has been required.

The research team at TMDU has found that "phosphonic acid dithioesters" are suitable starting materials for their purpose. These compounds showed high stability toward water and were able to be purified by silica-gel chromatography without special care. Moreover, phosphonic acid dithioesters showed an appropriate reactivity in the sequential reaction with two different Grignard reagents to afford various tertiary phosphine oxides in high yields.

"The key point of the novel method is the moderate leaving ability of a sulfur atom," the first author Yoshitake Nishiyama says. He explains: "The starting materials with phosphorus-chlorine bonds used in the conventional methods are generally highly reactive, but unstable instead. The starting materials with phosphorus-oxygen bonds are very stable, but in that case not reactive enough. Phosphorus-sulfur bonds in the starting materials and intermediates that appear in this method have been found sufficiently stable and also reactive enough toward Grignard reagents."

Using phosphonic acid dithioesters with stable phosphorus-sulfur bonds has rendered the research group able to achieve the synthesis of complex organophosphorus compounds in a combination with a bromo-magnesium exchange reaction. For example, they have demonstrated efficient synthesis of organophosphorus compounds such as a 1,2-diphosphinobenzene derivative and a cyclic phosphinate, which are difficult to prepare by the conventional methods.

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"The new method has enabled preparation of various organophosphorus compounds from three simple starting materials," one of the corresponding authors Takamitsu Hosoya says. He adds: "This means that, in principle, a combination of a hundred type of each three materials could easily produce a million type of products, which would contain candidates for pharmaceuticals or other useful materials."

The article "Synthesis of Unsymmetrical Tertiary Phosphine Oxides via Sequential Substitution Reaction of Phosphonic Acid Dithioesters with Grignard Reagents" was published in Organic Letters (Yoshida and Hosoya et al., Org Lett, 19: 3899-902, 2017).

Summary: TMDU researchers have developed a facile synthetic method for phosphine oxides bearing three different substituents. The choice of phosphonic acid dithioesters with appropriate chemical stability and reactivity as the starting materials was the key for success. Using this method, a wide range of potentially useful organophosphorus compounds have become easily available.

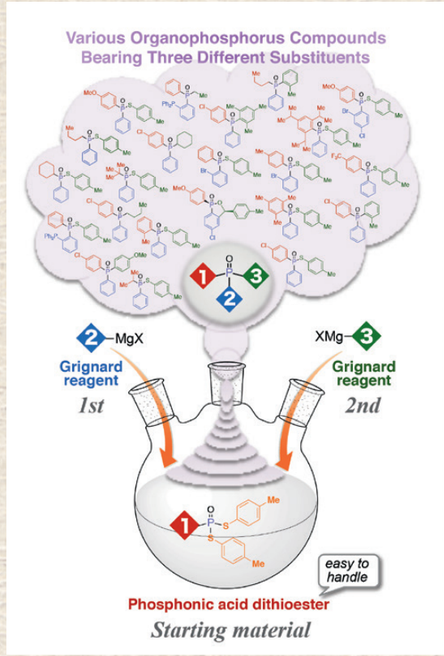

Fig.1: **Facile Synthesis of Various Tertiary Phosphine Oxides from Three Simple Starting Materials.**