‘Micro’ organism, ‘macro’ problems

Studies of the MERS-CoV macro domain conducted by NTU structural biologists reveal a promising new therapeutic target.

The macro domain is a well-known protein module with an affinity for ADP-ribose. RNA viruses such as severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV encode macro domains in their genomes, and the structures of several viral macro domains have been reported. However, the critical piece of the puzzle for the molecular structure and functional relationship of the viral macro domain remains missing.

SARS infamously emerged and resulted in 774 deaths in 2003. Ten years later, another potentially deadlier disease called MERS, also known as camel flu, has caused over 400 deaths since the first confirmed case was reported in Saudi Arabia in 2012. MERS is caused by a coronavirus that closely resembles the SARS virus but exhibits a much higher mortality rate; 40% of patients infected with MERS die, compared to 10% of patients infected with SARS. An effective treatment that cures MERS, an emerging disease that was recently discovered in 2012, is not available. Supportive care, including organ support to prevent complications, organ failure and secondary infections, remains the main treatment for MERS. Although many inhibitors targeting viral components that play critical roles in MERS-CoV replication have been reported, most of them are still in the early phases of investigation. The application of neutralizing monoclonal antibodies against MERS-CoV requires a high investment and rigorous testing and must undergo an approval process. Combinations of antivirals, interferons and corticosteroids have been used to treat patients infected with MERS, but none of them have resulted in a significant effect on clinical outcomes. Notably, the major challenge facing clinicians is the lack of specific anti-viral drugs with proven efficacy toward MERS.

Professor Chun-Hua Hsu’s research team from the Department of Agricultural Chemistry and the Genome and Systems Biology Degree Program has recently focused on understanding the structural features of these functionally diverse macro domains from various microorganisms. A conserved macro domain near the N-terminal region of non-structural protein 3 with unknown function was identified using bioinformatics analysis. According to Mr. Chao-Cheng Cho, a Ph.D. candidate from the Hsu lab, “Since MERS-CoV is a newly identified virus, we are curious about the existence of structural and functional divergences between macro domains from MERS-CoV and other CoVs”.

The macro domain is a protein module comprising approximately 180 amino acids that bind to the ester ADP-ribose to regulate cellular processes such as DNA repair, gene expression and controlled cell death. Recently, the macro domain from SARS-CoV, a coronavirus related to MERS-CoV, has been reported to suppress host immunity through

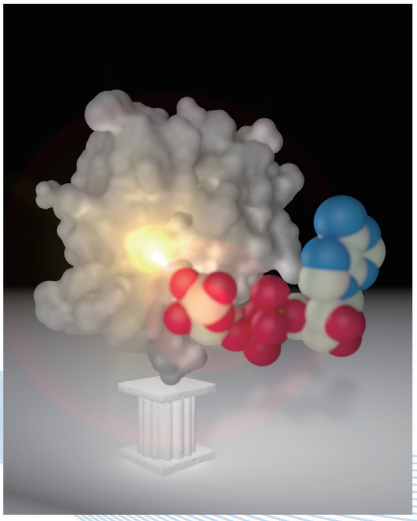

Figure 1. Image of an important protein structure called the macro domain (white surface) from the Middle East respiratory syndrome (MERS) coronavirus. The MERS macro domain binds to ADP-ribose (red and blue spheres) with high efficiency. Credit: 2016 Chun-Hua Hsu, National Taiwan University

its de-mono ADP-ribosylation activity. Using structural and biochemical approaches, Hsu's team discovered that MERS-CoV has a higher binding affinity for ADP-ribose than the macro domains from the CoVs that have been characterized to date. An interesting question has been raised: Does the higher binding affinity of the MERS-CoV macro domain for ADP-ribose account for the four-fold higher mortality rate observed in patients infected with MERS-CoV than in patients infected with SARS? This question will be answered in further investigations of the roles of the macro domain in MERS-CoV infection.

Professor Hsu's lab will conduct structure-based screens for potent inhibitors of the MERS-CoV macro domain using known drug databases and the traditional Chinese medicine database and will attempt to elucidate the inhibitory mechanism using biophysical techniques.

Reference
Chao-Cheng Cho, Meng-Hsuan Lin, Chien-Ying Chuang, and Chun-Hua Hsu (2016). Macro Domain from Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Is an Efficient ADP-ribose Binding Module: CRYSTAL STRUCTURE AND BIOCHEMICAL STUDIES. *Journal of Biological Chemistry, 291*(10):4894-4902. DOI:10.1074/jbc.M115.700542.

Professor Chun-Hua Hsu
Department of Agricultural Chemistry
anyhsu@ntu.edu.tw

Quadruplex formation enhanced by DNA methylation

Expression of the human telomerase reverse transcriptase gene is modulated by quadruplex formation in its first exon due to DNA methylation

It is well known that DNA secondary structure and methylation are involved in regulating gene expression in mammals. Research has shown that G-quadruplex (G4) structures, which are among the most prevalent non-B DNA structures, are key players in the control of gene transcription and regulation. Methylation of cytosine in CpG dinucleotides in promoter regions is a well-characterized epigenetic modification that plays an important role in regulating numerous cellular processes, including development and tumorigenesis. CpG dinucleotides are often found within potentially G4-forming sequences in the promoter regions of numerous genes. Although early research indicated that CpGs within higher-order G4-forming DNA motifs undergo low methylation, methylation of cytosines in CpG dinucleotides within these G4 motifs nonetheless occurs in the genome. However, the interplay between DNA methylation and DNA secondary structures and the resulting effects on the regulation of gene expression have not been addressed.

Our study began from the finding that oxidative stress mediated by photodynamic therapy (PDT) and chemotherapy can down-regulate the expression of human telomerase reverse transcriptase (hTERT). hTERT is the major component of the catalytic subunit of telomerase and acts as a rate-limiting factor for telomerase activity. Telomerase activity is observed in approximately 90% of human cancers, whereas most somatic normal tissues are negative for hTERT expression. Aberrant hTERT gene expression may cause aging, cancer, and other diseases. Several transcription factors with binding sites in the promoter region have been documented to directly or indirectly regulate