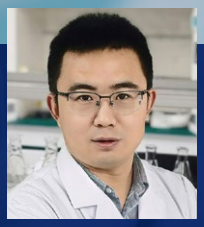
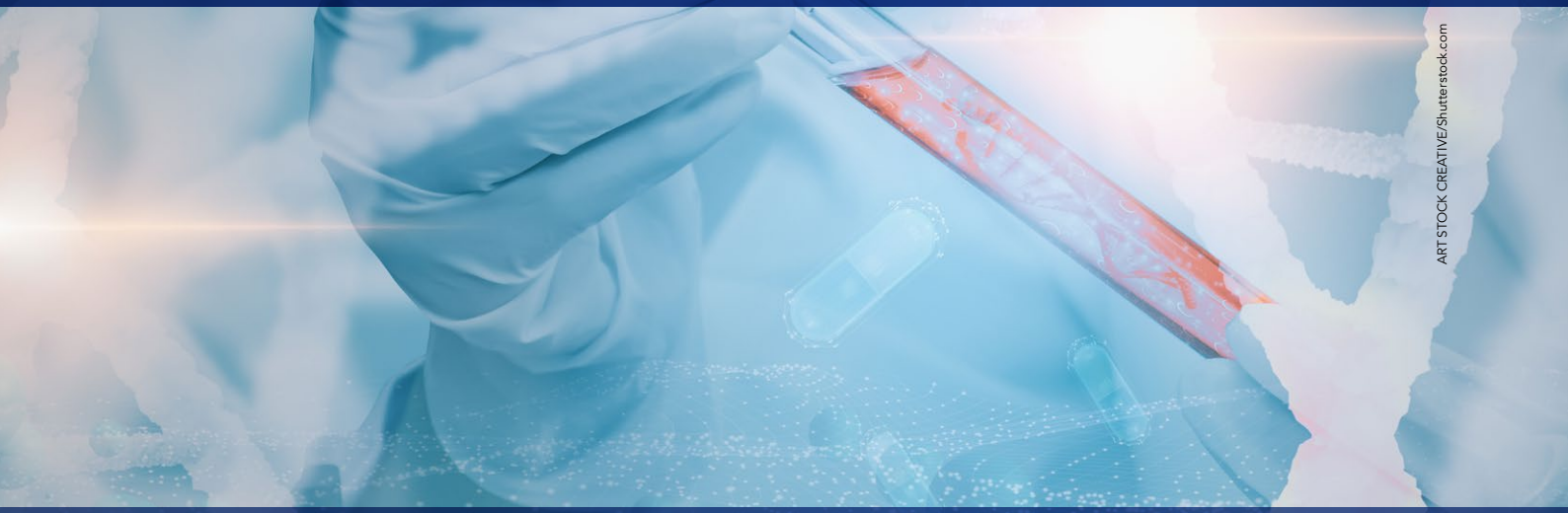


Dr Xinjie Wang

E: wangxinjie@caas.cn

Dr Peixiang Ma

E: peixiangma@outlook.com



ART STOCK CREATIVE/Shutterstock.com

CRISPR-based breakthrough for infectious disease detection and cancer diagnostics

Research Objectives

Wang and Ma have developed new, sensitive, and fast diagnostic tests for the detection of cancers and viral infections.

Detail

Address
Dr Xinjie Wang
Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

Dr Peixiang Ma
Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Acknowledgements
We thank all collaborators for extensive discussions and valuable suggestions. We thank the support from the Emergency Key Program of Guangzhou Laboratory (EKPGL2021018) and Innovation Research Funding from AGIS.

Agricultural Genomics Institute at Shenzhen
Chinese Academy of Agricultural Sciences

References

Liu, Y, Chen, Y, Dang, L, et al, (2021) EasyCatch, a convenient, sensitive and specific CRISPR detection system for cancer gene mutations. *Mol Cancer*, 20, 1–7. www.doi.org/10.1186/s12943-021-01456-x

Meng, Q, Wang, X, Wang, Y, et al, (2021) Detection of the SARS-CoV-2 D614G mutation using engineered Cas12a guide RNA. *Biotechnology Journal*, 16(6), 1–7. www.doi.org/10.1002/biot.202100040

Ma, P, Meng, Q, Sun, B, et al, (2020) MeCas12a, a highly sensitive and specific system for COVID-19 detection. *Advanced Science*, 7(20), 1–9. www.doi.org/10.1002/advs.202001300

Wang, X, Ji, P, Fan, H, et al, (2020) CRISPR/Cas12a technology combined with immunochromatographic strips for portable detection of African swine fever virus. *Communications Biology*, 3, 1–8. www.doi.org/10.1038/s42003-020-0796-5

Wang, X, Zhong M, Liu, Y, et al, (2020) Rapid and sensitive detection of COVID-19 using CRISPR/Cas12a-based detection with naked eye readout, CRISPR/Cas12a-NER. *Science Bulletin*, 65(17), 1436–1439. www.doi.org/10.1016/j.scib.2020.04.041

Personal Response

At which diagnostic stage(s) of cancer could CRISPR-Cas12a-based assays be used, and what other potential applications do you see for your technique?

"These assays can be used for newly diagnosed patients and recurrent patients. They are also applicable for patients who need precision-medicine companion diagnostics, such as target antibody or target chemical treatment."

CRISPR-based breakthrough for infectious disease detection and cancer diagnostics

CRISPR diagnostics are extensively used in the detection of genetic material. Dr Xinjie Wang at the Chinese Academy of Agricultural Sciences, Shenzhen, and Peixiang Ma at Shanghai Jiao Tong University School of Medicine, Shanghai, have developed new diagnostic tools using CRISPR-based technology, which are faster, more sensitive, and more accessible than existing diagnostic methods for infectious diseases. Importantly, Wang and Ma's assays also have potential applications for cancer diagnostics and the detection of drug-resistant mutations in cancer cells.

The importance of infectious disease detection became evident during the COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2, which resulted in the death of millions of people worldwide. One of the highest priorities was to establish a sensitive method to detect the virus quickly and reliably. Developing new, more sophisticated tests for cancer is also ranked high on the global healthcare agenda. Meeting this demand, Dr Xinjie Wang at the Chinese Academy of Agricultural Sciences, Shenzhen, and Peixiang Ma at Shanghai Jiao Tong University School of Medicine, Shanghai, developed CRISPR-based detection tools for infectious disease detection and cancer diagnostics. They have developed precise and accessible CRISPR-powered diagnostic assays that offer distinct advantages over conventional tests.

CRISPR/CAS: HOW IT WORKS

CRISPR is short for Clustered Interspaced Short Palindromic Repeat. CRISPR-based detection is used to track and identify a specific part of genetic material (DNA or RNA sequences). CRISPR can also be used to edit genomes (ie, alter parts of genetic material). It's considered to be one of the most powerful techniques used in research, superior to existing methods such as the real-time quantitative reverse transcription-polymerase chain reaction (qPCR), which is commonly used to detect SARS-CoV-2. qPCR requires expensive instruments and trained operators, struggles to detect certain mutations, and takes hours to produce a result. In comparison, CRISPR offers fast results, can be incorporated into portable diagnostic devices, and does not need expensive equipment. Since sensitivity and fast results are key to diagnostic effectiveness, Wang and Ma harnessed the valuable tool of CRISPR to develop new tests for infectious disease detection and cancer diagnostics.

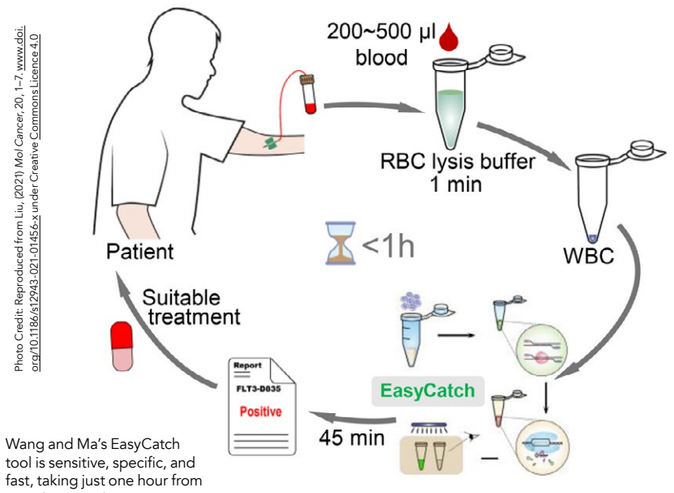


Photo Credit: Reproduced from Liu, (2021) *Mol Cancer*, 20, 1–7. www.doi.org/10.1186/s12943-021-01456-x under Creative Commons Licence 4.0

Wang and Ma's EasyCatch tool is sensitive, specific, and fast, taking just one hour from sample to results.

One of CRISPR's main applications is for detecting genetic material, using specific proteins called CRISPR-associated (Cas) protein. Cas proteins are used by bacteria to destroy viral genetic material; they add parts of the viral genetic material to their own genome to guide Cas protein toward the virus, and the proteins then excise the viral genes. Scientists have found a way to use this CRISPR-Cas technology to create unique diagnostic tools, which guide Cas protein towards the target genetic material. If the target material is present, the Cas protein releases specific molecules which act as visible identification signals.

FAST RESULTS TO THE NAKED EYE

Wang and Ma used the Cas12a protein to design an assay to detect infectious diseases which uses fluorescence (CRISPR-Cas12a-NER) or lateral flow strip (CRISPR/Cas12a-LFD) as an identification signal. This assay is very sensitive and fast: it can detect as few as ten copies of a virus gene in less than 45 minutes, and the results can be seen with the naked eye without a microscope. The method uses a fluorescent molecule that emits a green fluorescence when Cas12a identifies the desired viral sequence; at under 485nm light illumination, the fluorescence can be seen by the naked eye. To validate the assay, the researchers tested clinical samples using both the CRISPR-Cas12a-based assay and the World Health Organization-approved qPCR: the results of CRISPR-Cas12a-NER agreed 100% with those of qPCR.

Roman Zaiets/Shutterstock.com

CRISPR/Cas-based diagnostics have been used to detect a variety of pathogenic viruses and cancer mutation genes.

CRISPR/Cas-based diagnostic technology has been successfully applied to detect a variety of highly pathogenic viruses, including Zika (ZIKV), and papillomavirus (HPV). Wang and Ma are the first to use CRISPR/Cas12a to detect the African swine fever virus (ASFV). They proposed a molecular detection system targeting viral protein p72, using a combination of CRISPR/Cas12a technology and lateral flow strip, which they named ASFV CRISPR/Cas12a-LFD.

SENSITIVE AND SPECIFIC

One major drawback of several diagnostic methods, including Cas12a-based methods and the PCR, is the lack of test sensitivity – ie, these tests can't detect very small amounts of genetic material in a sample. To address this, Wang and Ma have designed a system called MeCas12a, which uses manganese to enhance the sensitivity of the CRISPR-Cas12a method. It's known that divalent cation ions, such as magnesium (Mg) and manganese (Mn), play a major role in Cas activity, and Wang and Ma successfully used Mn to increase the signal strength by up to 13 times. In addition, CRISPR/Cas12a was able to distinguish between different coronaviruses with remarkable accuracy. The researchers proved that MeCas12a can identify the mono or co-infection of SARS-CoV-2 and MERS-CoV (Middle East respiratory syndrome coronavirus) that caused an epidemic in 2012.

Another major disadvantage of current COVID-19 diagnostic tools is their inability to detect viral genetic material in cases of mutations, several of which have occurred in the viral genome during the evolution of SARS-CoV-2. Most of these mutations have been observed in the spike protein of the virus, such as in the case of the G614 variant; this strain is more infectious, making its detection an absolute necessity. To address this issue, Wang and Ma developed a new tool, symCRISPR/Cas12a, which was able to distinguish the G614 variants with impressive specificity.

EASYCATCH: RAPID AND ULTRASENSITIVE TUMOUR DETECTION

The CRISPR/Cas12a method offers ultrasensitive and fast tumour detection. Wang and Ma have developed the first tumour detection method that is simple, highly sensitive, specific, and fast. During drug therapy, cancer cells can mutate to evolve immunity to treatment and then develop into a drug-resistant tumour. One solution is to detect the mutation as early as possible and apply mutation-specific drugs. However, conventional methods, such as qPCR, next generation sequencing (NGS) and first generation sequencing (FGS), can only detect 0.1% of mutating cancer cells, or one mutation out of every 1,000 cells. The researchers' new method, called EasyCatch (Excision-amplification-synchronous Cas12a-targeted checkout), is more sensitive than any other current method, being able to detect one mutation in 100,000 cells. The EasyCatch method results in background signals from non-mutated cells being silenced so the signal produced by the mutation is amplified. The researchers demonstrated significant improvements in sensitivity and specificity of EasyCatch in detecting mutations for acute myeloid leukaemia, by benchmarking its results against the commonly used FGS and NGS. They have also shown it to be effective in the detection of other cancers, including glioma, lung cancer, and colorectal cancer. Another unique characteristic is the speed with which EasyCatch produces results: it takes just one hour from the blood sample being taken to results being available.

EasyCatch takes just one hour from the blood sample to results.

NEXT-GENERATION DIAGNOSTICS

The development of new tools, for sensitive and fast diagnosis, is crucial for the future of healthcare, and the disadvantages of existing methods are pushing the scientific community to find more accurate diagnostic methods. Wang and Ma's pioneering work paves the way for a new era of highly sensitive CRISPR-based diagnostics that are more accessible and deliver fast results. Overcoming the obstacles of predecessors, their unique methods are an invaluable breakthrough for infectious disease detection and cancer diagnostics.

researchfeatures.com

Research Features.

Complex science made beautifully accessible

researchfeatures.com

Partnership enquiries: simon@researchfeatures.com