A powerful new plant-based weapon against cancer

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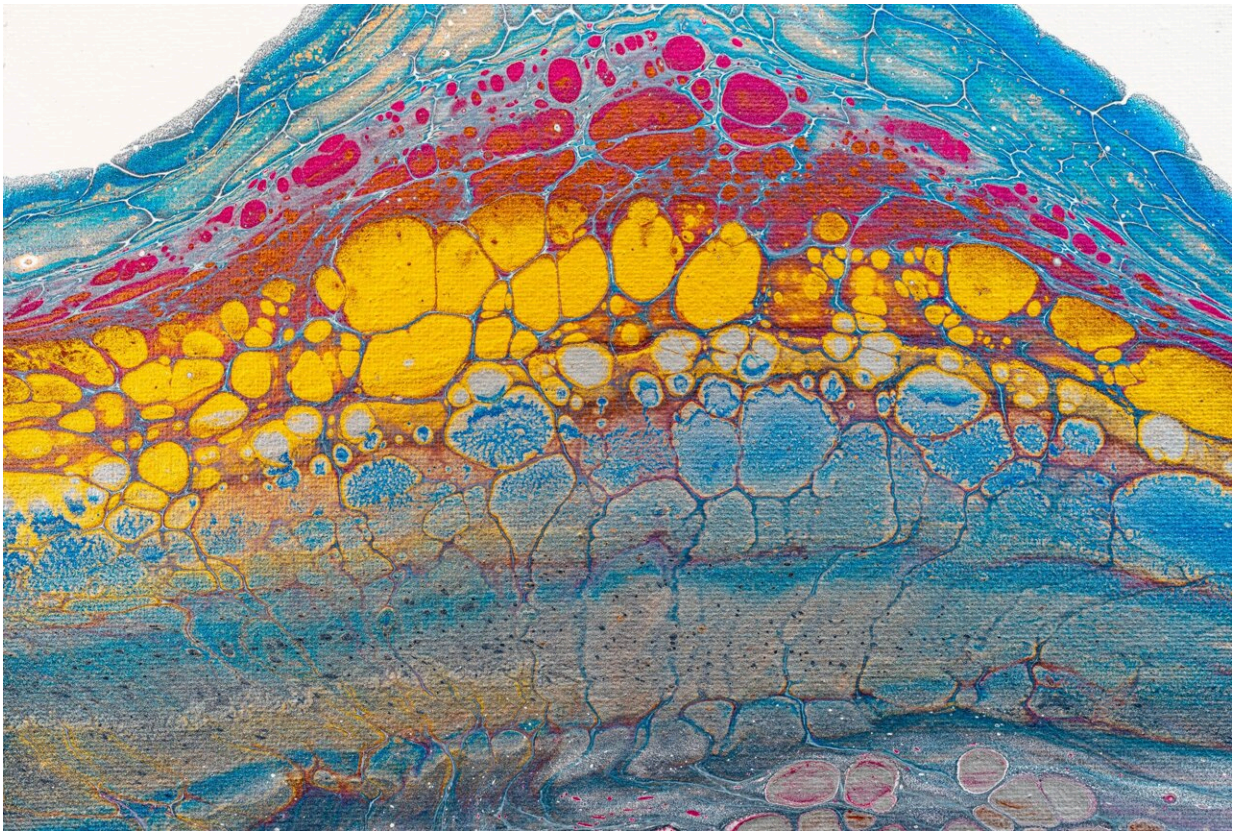

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Cancer is caused by abnormal cell proliferation and is one of the main public health issues in the world. Recently, the research group led by Researcher Du Peng of PKU School of Life Sciences discovered that a

plant immune protein enables broad anti-tumor response by alleviating micro-RNA deficiency, which provides a powerful weapon against cancer.

The causes of cancer

Micro-RNA(miRNA) has been considered closely related to carcinogenesis. Mammalian mature miRNA double stranded with 2-nt 3' terminal overhang can be recognized and loaded by Argonaute (AGO) to form RNA Induced Silencing Complex (RISC) to regulate the expression of target genes. In fact, dose reduction of global miRNA is considered to be one of the causes of cancer.

Over activation of cell cycle is a necessary condition for abnormal proliferation of cancer cells. Surprisingly, many miRNAs can directly target and inhibit cell cycle genes to control cell proliferation. Therefore, it would be a new strategy for tumor treatment to inhibit tumor cell proliferation by repairing the defective miRNA pathway in tumors.

Different from miRNA, siRNA is derived from double-stranded RNA substrates synthesized by different RNA-dependent RNA polymerases (RDR). RDR1-dependent siRNA in plants is one of the unique core molecular immune response pathways especially involved in antiviral immune response. Hence, Prof Peng's team aims to carry out plant genetic engineering based on plant RDR1 in mammals from the difference between animal and plant immune systems, and study its application in translational medicine.

On May 26, 2022, Prof Du Peng's research group published a research paper titled "A Plant Immune Protein Enables Broad Antibody Response by Recovering Micro-RNA Deficiency" in *Cell*, which introduces that miRNA isomers that cannot effectively bind to the 1-nt-shorter 3′ends of the AGO2 complex are widely accumulated in different samples of

human primary cancers and cancer-cell lines. RDR1, as ectopically expressed plant immune protein, modifies these double stranded free miRNA isomers of AGO2 through its single nucleotide tailing, to reactivate the defective miRNA pathway and specifically block the cycle of cancer cells in solid tumors and leukemia.

Exciting Results of Research

The researchers made four major discoveries:

Firstly, RDR1 protein inhibits the proliferation of cancer cells by targeting cell cycle. The authors cloned RDR1 gene respectively from Arabidopsis (At) and rice (Os) into lentivirus vector induced by Dox, and verified its successful ectopic expression in mammalian cells. At the molecular level, Gene Set Enrichment Analysis (GSEA) based on RNA-seq showed that RDR1 in At and Os could interfere with cycle processes in all cancer cell lines, while it had no significant effect in non-cancer control cells. The authors believe that RDR1 is an exogenous tumor suppressor which can specifically target and interfere with the cycle process in cancer cells without influence on non-cancer cells. RDR1 in At and Os has broad-spectrum and specific inhibition of cancer cell proliferation, while having no effect on non-cancer cell lines.

Secondly, 3'- terminal short 1-nt miRNA isomers are widely accumulated in a variety of tumors. The authors proposed that plant RDR1 can inhibit cell cycle and proliferation by increasing global miRNA expression to specifically recover miRNA deficiency in cancer cells through knockdown and AGO2-CLIP of key components of miRNA pathway. And through systematic analysis on the published miRNA sequencing data and the miRNA sequencing of AGO2-IP, the authors suggest that it is not so effective and stable for the abnormal short 1-nt double-stranded miRNA isomer to enter into AGO2 in cancer cells, so it may be related to the reduction of miRNA dose in different

tumors. Thirdly, RDR1 repairs miRNA isomers in cancer by single nucleotide tailing. Through biochemical experiments in vitro, the authors directly proved that rAtRDR1 could modify single-stranded miRNA and double-stranded miRNA with 1-nt or 2-nt overhang by 3' terminal single nucleotide, but could not modify double-stranded miRNA with flat ends. The authors also proved that RDR1 with nucleotide transferase activity can modify the short 1-nt double-stranded miRNA isomers isolated from AGO2 by single nucleotide, so as to restore their loading efficiency to AGO2, and finally repair the defective miRNA pathway in cancer. Lastly, RDR1 inhibits the progression of various mouse solid tumors and leukemia. The authors verified the anti-tumor effect of plant RDR1 in mouse models with immunodeficiency and within vivo leukemia. Finally, RDR1 protein purified in vitro by nano vesicle package and AAV packaged respectively achieve direct delivery and tumor inhibition at the level of cells in vitro and solid tumors in vivo.

The study reveals for the first time that abnormal 3' terminal short 1-nt miRNA isomers are widely accumulated in various human primary tumors, which provides a new insight into the reduction of global miRNA dose during tumorigenesis. Using RDR1, we achieved a broad-spectrum anti-tumor response by repairing miRNA defects in cancer cells, and developed a new strategy to edit and manipulate miRNA, making it a powerful weapon against cancer.

More information: Ye Qi et al, A plant immune protein enables broad antitumor response by rescuing microRNA deficiency, *Cell* (2022). DOI: 10.1016/j.cell.2022.04.030

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