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Research Highlight

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CRTH2: a potential target for the treatment of organ fibrosis

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Fibrosis, the self-repair process of the body after tissue damage, is a common pathological feature of many chronic inflammatory diseases. It occurs in a variety of organs, leading to structural destruction and hypofunction, and even organ failure. Fibrosis in organ tissues is characterized by chronic inflammation, excessive accumulation of extracellular matrix (ECM) components, and the decrease of parenchymal cells. Fibrosis is caused by persistent infections, toxins, autoimmune diseases, radiation, and mechanical injury. To date, there are various therapeutic strategies for organ fibrosis, such as changing lifestyle, using antiviral drugs, anti-inflammatory treatment, using interferon, and organ transplantation. Currently, there are only two FDA-approved drugs for the treatment of idiopathic pulmonary fibrosis (IPF), i.e., pirfenidone and nintedanib. Nevertheless, the mechanisms of action of these drugs are not fully understood, the cost is high, and there is no way to prevent or reverse the disease process. Consequently, it is extremely important for us to better understand the pathogenesis of fibrosis, strengthen the screening and evaluation of new drugs, and improve the existing drugs. And it can also help us to treat and prevent fibrosis in the future.

Fibroblasts and differentiated myofibroblasts produce abundant ECM in organ fibrosis, and the main component is collagen. The biosynthesis of collagen is relatively complicated and is precisely regulated at multiple levels, including mRNA transcription, splicing of mRNA, mRNA stability, mRNA translation, protein post-translational modification, and protein degradation. The abnormal increase in collagen expression during organ fibrosis is mainly due to the increased stability of collagen mRNA and its translation. An evolutionarily conserved stem-loop (SL) structure exists in the 5′ untranslated regions (5′UTRs) of type I and type III collagen mRNAs, which bind to the RNA recognition motif (RRM) domain of La ribonucleoprotein domain family member 6 (LARP6) to regulate the stability of collagen mRNA and its translation [1,2].

The chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), also known as DP2, is a G-protein-coupled receptor (GPCR) with seven transmembrane helical domains and acts as a cell membrane receptor for prostaglandin D2 (PGD2). CRTH2 is initially found in type 2 helper T (Th2) cells and is highly expressed in innate lymphocytes, eosinophils, and basophils. CRTH2 is a highly conserved protein among different species, indicating that it plays a crucial role in physiological and pathological processes (Figure 1A). CRTH2 mediates numerous functions of immunocompetent cells, such as allergic inflammation, pro-inflammatory functions, and anti-inflammatory effects. The PGD2-CRTH2 signaling pathway mediates type 2 inflammatory responses, which results in the activation of immune cells and the production of type 2 cytokines. This signaling pathway has become one of the most promising therapeutic targets in the field of allergy and asthma [3]. It also has a crucial effect on cardiovascular diseases. Zuo et al. [4] unveiled that inhibition of CRTH2 can restrain the apoptosis of cardiomyocytes caused by endoplasmic reticulum (ER) stress and has a potential therapeutic effect on apoptotic cardiomyopathy. Meanwhile, Chen *et al.* [5] confirmed that the lack of CRTH2 suppresses Th2 immune responses in the lungs, reducing the development of hypoxia-induced pulmonary arterial hypertension in mice. In addition, CRTH2 is also involved in regulating other physiological functions, and the PGD2-CRTH2 signaling pathway plays a significant role in host defense against bee venom [6]. Central CRTH2 mediates emotional impairment in the lipopolysaccharide and tumor-induced sickness behavior model [7]. Furthermore, Ueda *et al.* [8] found that loss of CRTH2 clearly exacerbates fibrosis induced by bleomycin, implying that CRTH2-mediated responses protect against the fibrotic processes in the lungs.

Recently, Zuo *et al.* [9] further demonstrated that CRTH2 mediates organ fibrosis. They discovered that CRTH2 is not only located in the cell membrane of all tested cell lines, but also located in the ER membrane of some cell lines. Consequently, they speculated that CRTH2 in the ER may be trafficked from the cell membrane of fibroblasts. Caveolin-1 is associated with GPCR trafficking and is highly expressed in fibroblasts. When caveolin-1 is knocked down, the distribution of CRTH2 in ER is significantly reduced and the distribution of CRTH2 in plasma membrane is obviously increased. However, overexpression of caveolin-1 leads to the completely opposite phenomena. Their results suggested that caveolin-1 mediates the trafficking of CRTH2 from the plasma membrane to the ER membrane in fibroblasts. In addition, they used co-im-

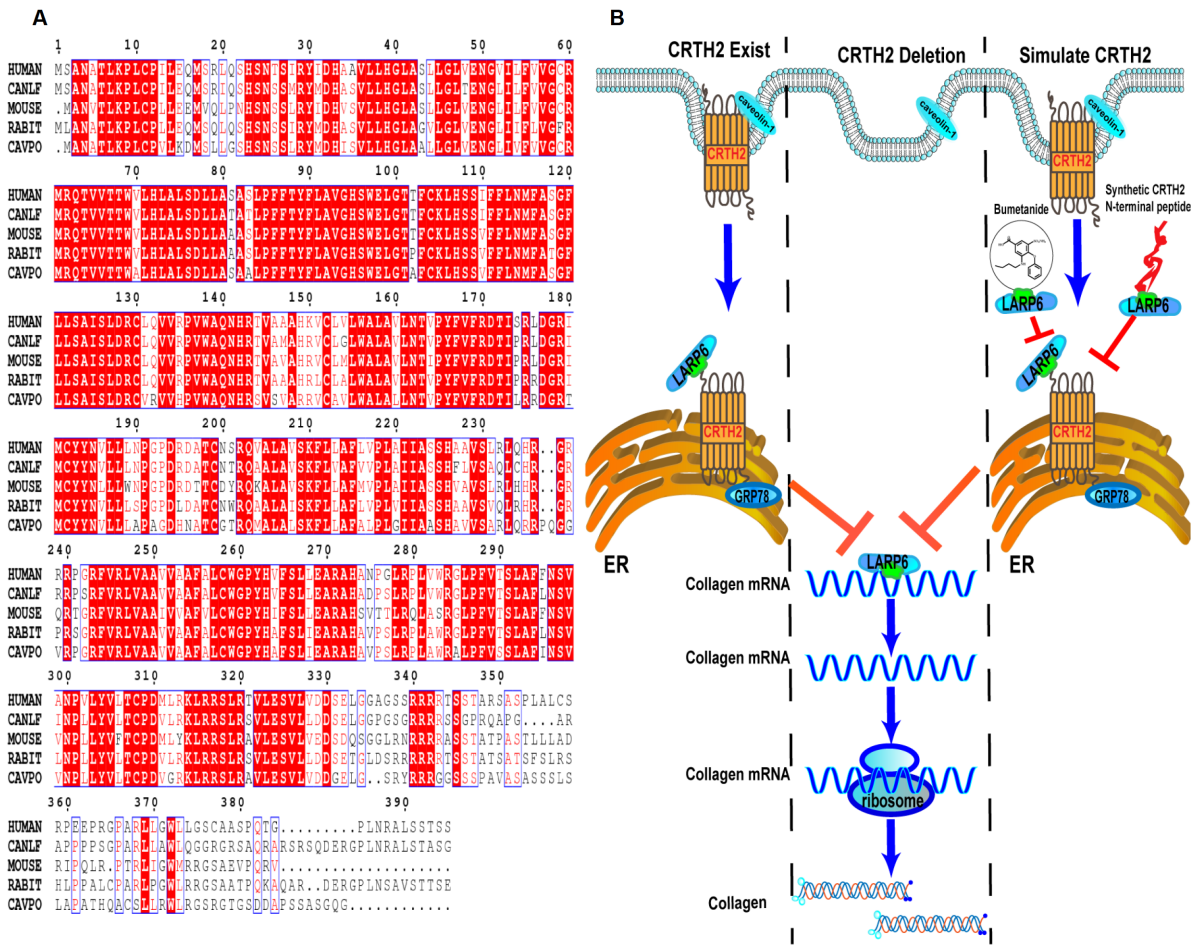

Figure 1. Comparison of CRTH2 amino acid sequences among different species and the mechanism of action of CRTH2 in inhibiting organ fibrosis
(A) Comparison of CRTH2 amino acid sequences among *Homo sapiens* (HUMAN), *Canis lupus* familiaris (CANLF), *Mus musculus* (MOUSE), *Oryctolagus cuniculus* (RABIT), and *Cavia porcellus* (CAVPO). The degree of conservation of CRTH2 amino acid sequences among different species was analyzed by ESPript software. The residues surrounded by blue rectangles are conservative. The residues in the red background are constant in different species. The similarity of the amino acid sequence of CRTH2 is 88.87% among different species. (B) The RRM domain of LARP6 binds to collagen mRNA and stabilizes it to promote collagen biosynthesis. The existence of CRTH2 promotes its trafficking from the plasma membrane to ER by caveolin-1. The C-terminal of CRTH2 binds to GRP78 and anchors to the ER, while the N-terminal of CRTH2 binds to the RRM domain of LARP6 to inhibit collagen biosynthesis. The synthetic CRTH2 N-terminal peptide and bumetanide simulate CRTH2 to bind to the RRM domain of LARP6, thereby inhibiting the binding of CRTH2 to LARP6 and inhibiting collagen biosynthesis.

munoprecipitation (co-IP) and mass spectrometry (MS) to show that CRTH2 interacts with LARP6 and GRP78. Subsequently, the authors constructed a number of truncated fragments in order to identify the structural regions in which the three proteins interact. The C-terminal of CRTH2 binds to GRP78 and anchors to the ER, while the N-terminal of CRTH2 binds to the RRM domain of LARP6 in the cytoplasm. However, LARP6 has a significant effect on the biosynthesis of collagen. Does CRTH2 have an impact on the biosynthesis of collagen? Firstly, when CRTH2 is deleted, the mRNA expression of collagen is increased, the cellular decay of mRNA collagen is inhibited, and the binding of LARP6 to mRNA collagen is promoted. However, these changes were attenuated by*LARP6* knockdown. Subsequently, Zuo and his colleagues established mouse models with the CRTH2 gene knockout (F-CRTH2 KO) and with the CRTH2 and LARP6 genes double knockout (F-DKO) in fibroblasts to observe the relationship between CRTH2 and organ fibrosis. Knockout of the CRTH2 gene significantly increases collagen biosynthesis and markedly aggravates the injury-induced fibrosis in the mouse parenchymal organs. Meanwhile, LARP6 gene deletion rescues the excessive fibrosis of the parenchymal organs of the CRTH2-knockout mice with different damages. Taken together, their results indicated that CRTH2 achieves the purpose of inhibiting collagen synthesis and inhibiting organ fibrosis by competing with collagen mRNA to bind to LARP6 (Figure 1B).

Is it possible to simulate the binding of CRTH2 with the RRM domain of LARP6 to treat organ fibrosis? Zuo and his colleagues synthesized a CRTH2 N-terminal peptide, and found that it can inhibit the binding of CRTH2 with LARP6 and significantly reduce the biosynthesis of collagen in fibroblasts. In addition, based on the large TargetMol bioactive compound library using the structure-based docking approach, collagen production inhibition assay, and microscale thermophoresis analysis, they discovered that bumetanide has a strong physical interaction with the RRM domain of LARP6 by simulating CRTH2. In a bleomycin-induced mouse model of pulmonary fibrosis, bumetanide mitigates pulmonary fibrosis and inhibits pulmonary fibrosis exacerbated by CRTH2 depletion. More interestingly, bumetanide inhibits the interaction between CRTH2 and LARP6, and suppresses the TGF-β-induced increase of

collagen mRNA and protein expressions in human fibroblasts.

Interestingly, CRTH2 can translocate across caveolin-1 from the plasma membrane to the ER membrane and redistribute in subcellular organelles. Previous studies have never found that CRTH2 on the plasma membrane has the function of binding with LARP6 to inhibit collagen synthesis. Remarkably, the ER is a huge membrane system that interacts not only with the nuclear membrane and the plasma membrane, but also with various organelles such as mitochondria and Golgi apparatus. Whether CRTH2 can be trafficked from the ER to the nuclear membrane or other organelles for redistribution and interact with other proteins remain to be further investigated to reveal new functions of CRTH2. In addition, the important role of CRTH2 in organ fibrosis makes itself be a novel promising therapeutic target. The N-terminal (1–32 amino acids) of CRTH2 is more conservative than the C-terminal (308–357 amino acids) (Figure 1A). Therefore, it is possible that the synthetic CRTH2 N-terminal peptides are better mimics for the binding of CRTH2 and LARP6. As we all know, bumetanide is a commonly used strong diuretic for the treatment of edema associated with congestive heart failure and kidney disease [10]. However, it may have new applications in inhibiting organ fibrosis, which provides an important basis for the new use of this old drug. Hence, the synthetic CRTH2 N-terminal peptides and bumetanide may provide new research directions and treatment strategies for the treatment of fibrosis in the future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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