Experimental strategy is the first to tackle fibrosis and scarring at the cellular level

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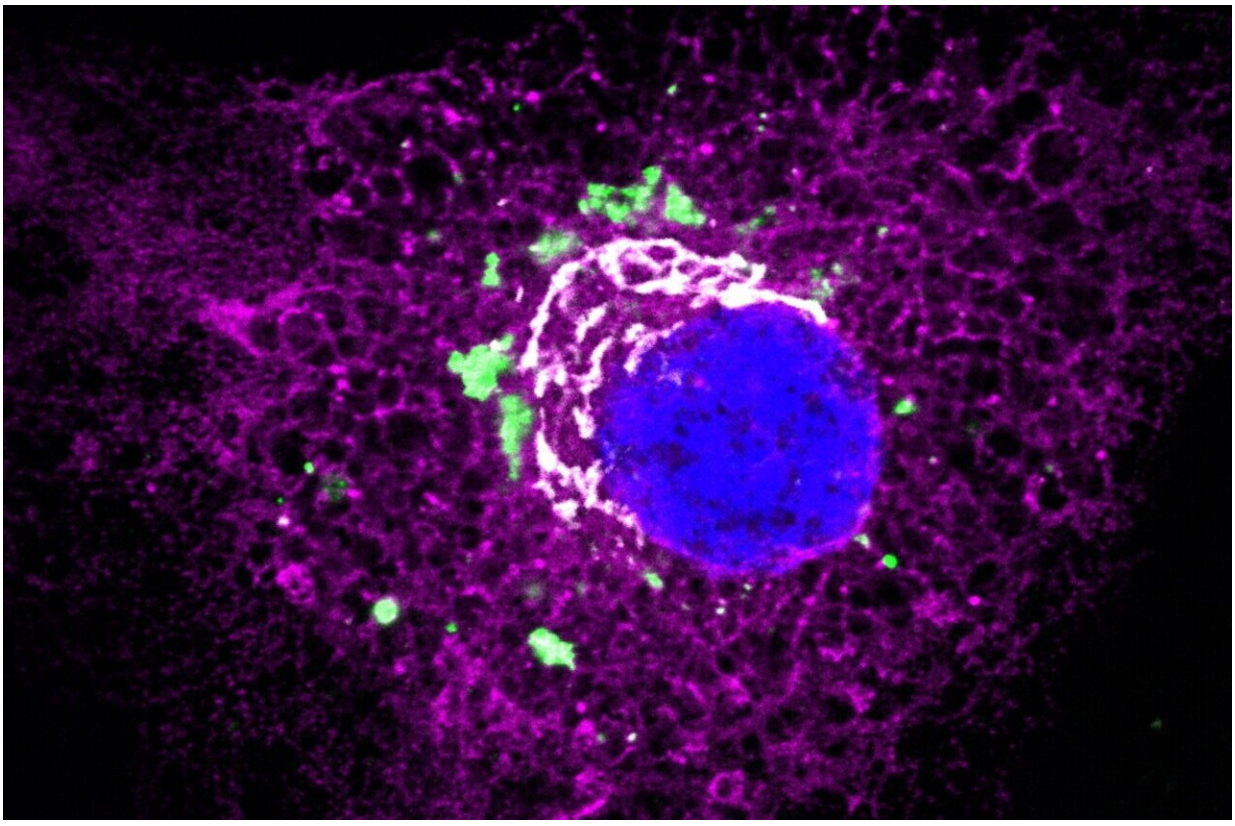

Peptide inhibitors bringing about collagen (shown in green) retention inside cells. Credit: Ishier Raote/Centro de Regulación Genómica

Researchers at the Center for Genomic Regulation in Barcelona and the University of Cologne in Germany have developed a new experimental strategy to tackle scarring and fibrosis. Experiments with patient-derived human cells and animal models showed the strategy was effective, non-toxic and its effects reversible. The findings are published in the journal *Nature Communications*.

Scarring occurs from the secretion and accumulation of various components—primarily proteins known as collagens—into the space between individual cells, usually occurring as a response to injury or damage.

Excessive collagen secretion can also cause the buildup of fibrotic tissue, a more serious condition where excess connective tissue is formed to the extent that it compromises the function of tissues and sometimes entire organs. Around 45% of deaths in the industrialized world are attributed to some form of tissue fibrosis.

Treatment options for both scarring and fibrosis are usually limited to surgery. Outside the body, scar tissue is often beneath the outer layer of the skin. Since most topical creams are not able to penetrate deeply enough to reach the affected areas effectively, their ability to remodel or heal the tissue is limited. Inside the body, scarring and fibrosis can affect many different tissues and organs, each with its unique environment and challenges and with no one-size-fits-all treatment option.

"Existing treatment options are usually ineffective because they try and fail to mop the excessive collagen up. In this work, we tried a completely different idea: to block the floodgates at the cellular level. The strategy works at the cellular level, releasing enough collagen so that tissues don't fall apart while protecting them from excessive amounts that impairs

their function," explains ICREA Research Professor Vivek Malhotra, co-corresponding author of the study and researcher at the Center for Genomic Regulation (CRG) in Barcelona, Spain.

The researchers' new strategy involves using molecules known as peptides to block the export of collagen from inside cells. The peptides disrupt an interaction between two proteins called TANGO1 and cTAGE5. Both proteins bind to each other and are essential for the export of collagens from their site of synthesis inside the cell to the exterior. The two proteins "sit" at the endoplasmic reticulum exit site, a place in the cell where materials like proteins are packaged and transported out the cell.

"Targeting the endoplasmic reticulum exit site has been historically considered impossible because a third of all human proteins go through it, so inhibiting its activity would likely have many off-target, toxic effects. In other words, it's been 'undruggable.' Only recently have there been indications that there is some specificity for the secretory materials. In this study we aimed to achieve this specificity by inhibiting the interface between TANGO1 and cTAGE5 with targeted precision," explains Dr. Ishier Raote, first author of the study who carried out the work at the Center for Genomic Regulation.

Proteins are like puzzle pieces. To know how two pieces fit together, you need to see their shapes clearly. Both TANGO1 and cTAGE5 are large, complex proteins which constantly shapeshift. To date, their exact structure remains unknown, which in turn means we don't understand how they connect at the molecular level, hindering efforts to design drugs that can block the interaction.

The researchers overcame this challenge by using AlphaFold2, an artificial-intelligence program that can guess the shapes of the two proteins without needing structural data about their 3D shape. The

predictions made by AI allowed the authors of the study to design peptides which can pass through a cell membrane and disrupt the interaction between TANGO1 and cTAGE5.

The researchers tested the peptides using normal human fibroblasts, the most common type of cell found in connective tissue. The peptides successfully inhibited collagen export, causing it to accumulate inside the cells. The effect was also reversible, with collagen levels increasing again after the peptides were removed within a 48-hour period.

The researchers observed similar effects in experiments with fibroblasts from patients with scleroderma, a complex autoimmune disease characterized by fibrosis of the skin and internal organs. The peptides were then tested using zebrafish, a common animal model to study tissue development and wound healing. The strategy visibly reduced collagen deposition in wound areas.

The researchers next plan to evaluate the efficacy of the peptides in pig skin because it closely resembles human skin. They will also finetune the properties of the peptides to increase their potency.

"We believe this represents a new strategy to control the effects of collagen hypersecretion. This could range from alleviating the cosmetic effects of skin scarring to the treatment of autoimmune diseases like scleroderma, as well as to manipulate post-surgery related events associated with wound healing to prevent fibrosis," concludes Dr. Malhotra.

More information: TANGO1 inhibitors reduce collagen secretion and limit tissue scarring, *Nature Communications* (2024). DOI: 10.1038/s41467-024-47004-1

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