Project develops develop new stem cell lines whose receptors can be activated by blue light

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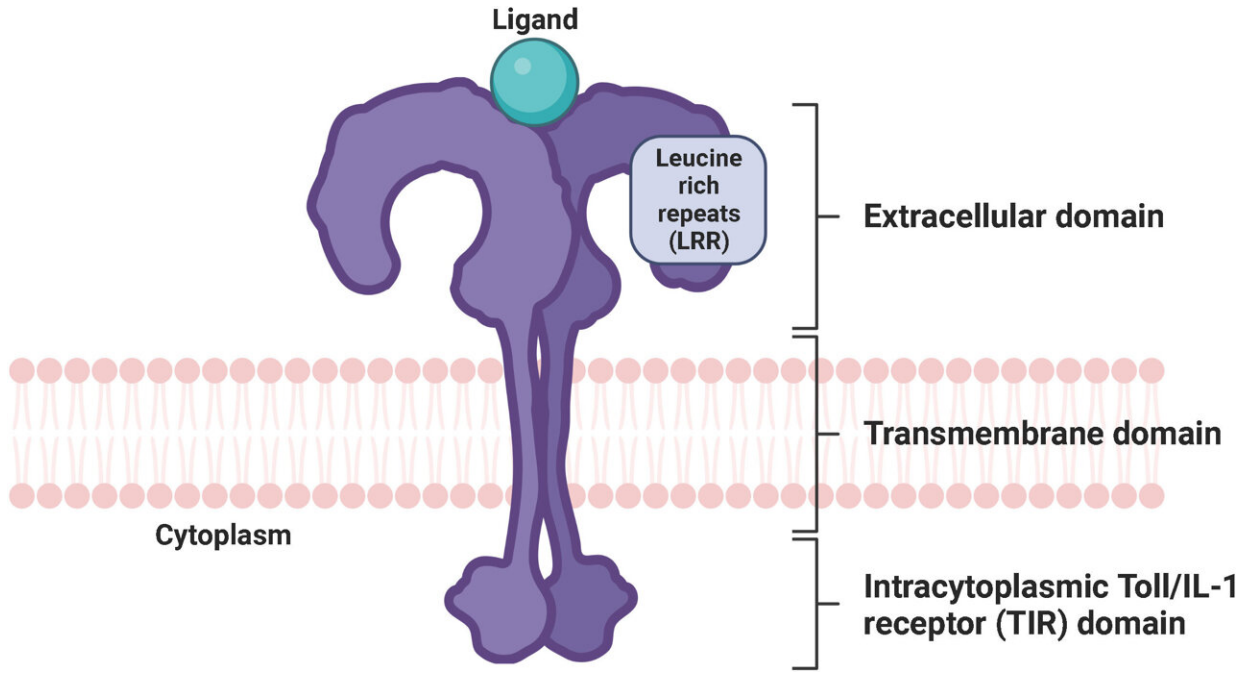

Conserved structure of TLRs. Credit: *Frontiers in Immunology* (2023). DOI: 10.3389/fimmu.2023.1264889

A research project led by the IMC University of Applied Sciences Krems has been successfully completed and provides an excellent basis for further projects. The team, led by Prof. Christoph Wiesner from the Institute of Biotechnology, has succeeded in genetically modifying special receptors (Toll-like receptors, TLRs) on stem cells so that they

can be activated by blue light.

Such "optogenetic" techniques could be used to precisely control biological signaling pathways in cells, validate them under physiologically relevant conditions and generate disease models. These new optogenetic cell lines will also make a valuable contribution to the understanding of disease mechanisms and the development of innovative, targeted therapeutic approaches.

For several years, the group led by Wiesner, holder of the "Cellomics / High Content Screening" research professorship at the IMC Krems, has been focusing on optogenetics—an emerging field of research that deals with the targeted control of cells using light.

The aim of the project, which has now been successfully completed, was to develop new stem cell lines (MSCs, mesenchymal stromal cells) whose receptors have been genetically modified by incorporating light-sensitive proteins so that they can be activated by blue light.

The work is published in the journal *Frontiers in Immunology* and International Journal of Molecular Sciences.

MSCs are all-rounders

"In our project," explains Prof Wiesner, "we worked with so-called MSCs, or mesenchymal stromal cells. These are adult stem cells that can be found in a variety of tissues and can differentiate into different cell types."

MSCs are found in the body in two different states (MSC1 and MSC2), which have different functions: The MSC1 cells have a pro-inflammatory effect, i.e. they promote inflammatory reactions and thus support the immune system to fight infections and tumors.

The anti-inflammatory MSC2 cells, on the other hand, dampen inflammatory responses in the body and are therefore useful in chronic inflammation, autoimmune diseases or to promote tissue repair after injury. It is known that all MSCs carry special receptors on their cell surface—TLRs—that recognize their molecular pattern on contact with pathogens and trigger an immune response via subsequent signaling pathways.

However, the exact mechanisms by which the activation of different TLRs leads to the development of the two forms of MSCs are still poorly understood—a fact that Prof Wiesner's research team has addressed.

A question of regulation

Based on the hypothesis that MSCs can take on different functions depending on the activated TLR type and the strength of the stimulus (e.g., pro- and anti-inflammatory, antibacterial or regenerative tasks), transgenic and optogenetic approaches should help to elucidate the mechanisms that lead to the polarization of MSCs into the two forms MSC1 and MSC2.

"To do this, we incorporated light-sensitive proteins into the TLRs so that we could switch the receptors on by light and off again by darkness," explains Prof. Wiesner.

In particular, it was shown that TLR4 and TLR10 could be easily controlled by light after incorporation into the cell lines. The following observations proved that the optogenetic constructs worked perfectly: TLR4 activation led to the production of pro-inflammatory molecules, similar to bacterial infection, while TLR10 activation regulated both pro- and anti-inflammatory molecules.

Extensive analysis of the supernatant of the cultured MSC cell lines revealed numerous proteins indicative of the cells' regenerative potential and accelerated bone cell formation after TLR10 activation. This makes the new cell lines useful tools for investigating the mechanisms of TLR4 and TLR10 activation and may provide new approaches for therapeutic strategies.

The ESPRIT project with Anna Stierschneider, Senior Postdoc in Christoph Wiesner's research group, in which miniaturized (0.2–0.5 mm in size), physiologically relevant 3D heterotypic cell models are established in vitro, shows that the new optogenetic cell lines can be tested in more than just individual experiments.

The optogenetic stem cells are integrated into heterotypic tumor cells (colorectal adenocarcinoma); 96 of these miniaturized tumors are cultivated in parallel and the optogenetic approach is tested for its anticarcinogenic potential. Initial experiments are promising.

The successfully completed project has not only resulted in these published studies, but also in further research projects and valuable collaborations—including the follow-up project ESPRIT and a training program for doctoral students (doc.funds), which the IMC Krems is coordinating with the University of Applied Sciences Krems and MedUni Vienna.

Together with ABS Biotechnology GmbH, a GFF application was submitted to introduce optogenetic constructs into inducible pluripotent cells and investigate their effects on cardiac myocytes and macrophages.

In collaboration with KL Krems and the UPEC University (Paris), the effect of TLR-activated mesenchymal stem cell supernatants on neurons and in Alzheimer's disease models will be investigated.

More information: Anna Stierschneider et al, Shedding light on the molecular and regulatory mechanisms of TLR4 signaling in endothelial cells under physiological and inflamed conditions, *Frontiers in Immunology* (2023). DOI: 10.3389/fimmu.2023.1264889

Katrin Colleselli et al, An Update on Toll-like Receptor 2, Its Function and Dimerization in Pro- and Anti-Inflammatory Processes, *International Journal of Molecular Sciences* (2023). DOI: 10.3390/ijms241512464

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