Streamlining stem cells to treat macular degeneration

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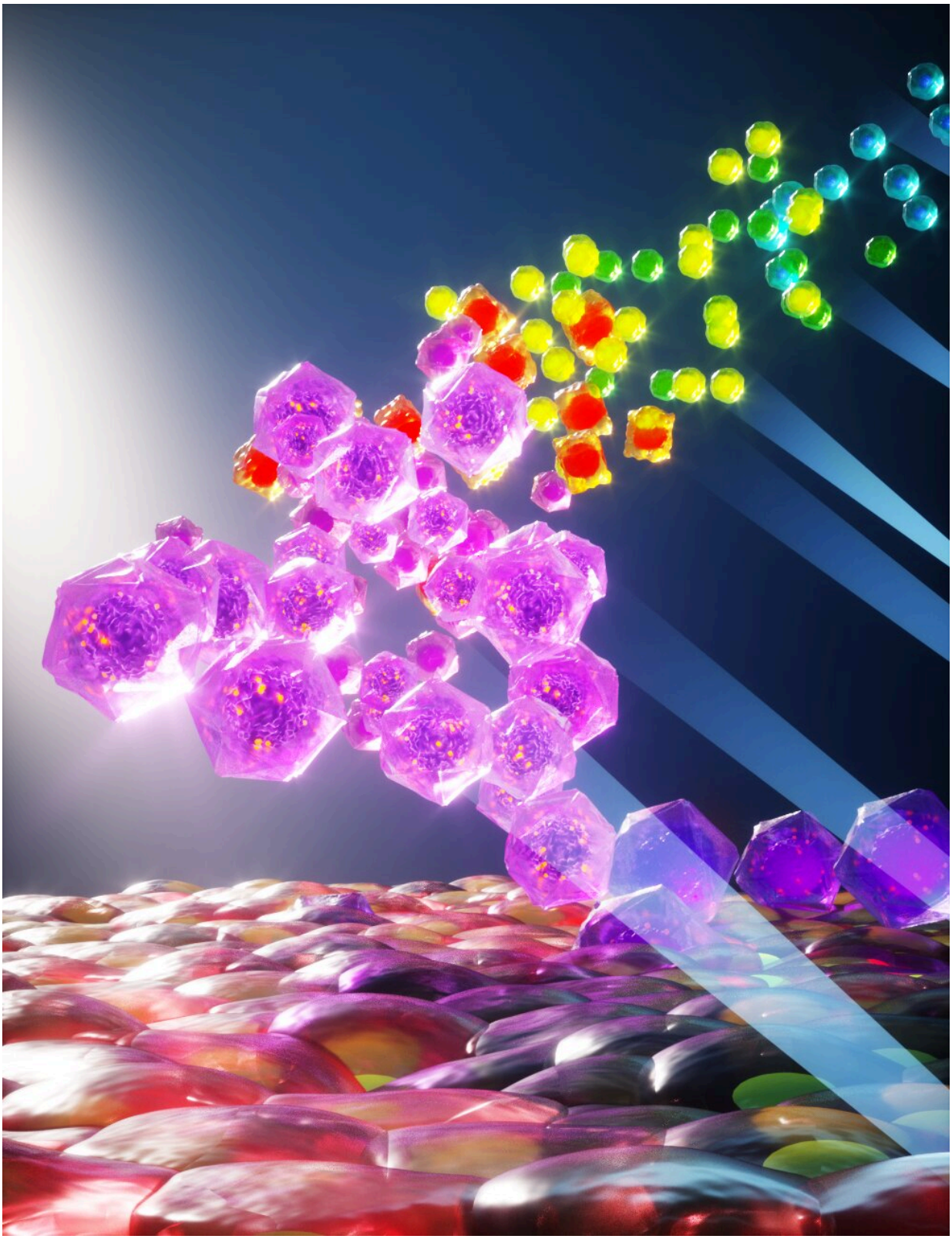

The differentiation of human embryonic stem cells into retinal pigment

epithelium for therapeutic use to treat acute macular degeneration. The "spotlights" represent the use of single-cell RNA sequencing to reveal the gene expression state of cells at early, mid, and late time points of retinal pigment epithelium differentiation. Credit: Ella Maru Studio.

As we age, so do our eyes; most commonly, this involves changes to our vision and new glasses, but there are more severe forms of age-related eye problems. One of these is age-related macular degeneration, which affects the macula—the back part of the eye that gives us sharp vision and the ability to distinguish details. The result is a blurriness in the central part of our visual field.

The macula is part of the eye's retina, which is the light-sensitive tissue mostly composed of the eye's visual cells: cone and rod photoreceptor cells. The retina also contains a layer called the retinal pigment epithelium (RPE), which has several important functions, including light absorption, cleaning up cellular waste, and keeping the other cells of the eye healthy.

The cells of the RPE also nourish and maintain the eye's photoreceptor cells, which is why one of the most promising treatment strategies for age-related macular degeneration is to replace aging, degenerating RPE cells with new ones grown from human embryonic stem cells.

Scientist have proposed several methods for converting stem cells into RPE, but there is still a gap in our knowledge of how cells respond to these stimuli over time. For example, some protocols take a few months while others can take up to a year. And yet, scientists are not clear as to what exactly happens over that period of time.

Mixed cell populations

"None of the differentiation protocols proposed for clinical trials have been scrutinized over time at the single-cell level—we know they can make retinal pigment cells, but how cells evolve to that state remains a mystery," says Dr. Gioele La Manno, a researcher with EPFL's Life Sciences Independent Research (ELISIR) program.

"Overall, the field has been so focused on the product of differentiation, that the path undertaken has been sometimes overlooked," he adds. "For the field to move forward, it is important to understand aspects of the dynamics of what happens in these protocols. The path to maturity could be as important as the end state, for example for the safety of treatment or for improving cell purity and reducing production time."

Tracking stem cells as they grow into RPE cells

La Manno has now led a study with Professor Fredrik Lanner at the Karolinska Institute (Sweden) profiling a protocol for differentiating human embryonic stem cells into RPE cells that is actually intended for clinical use. Their work shows that the protocol can develop safe and efficient pluripotent stem cell-based therapies for age-related macular degeneration. The study is published and featured on this month's cover of the journal *Stem Cell Reports*.

"Standard methods such as quantitative PCR and bulk RNA-seq capture the average expression of RNAs from large populations of cells," says Alex Lederer, a doctoral student at EPFL and one of the study's lead authors. "In mixed-cell populations, these measurements may obscure critical differences between individual cells that are important for knowing if the process is unfolding correctly." Instead, the researchers used a technique called single-cell RNA sequencing (scRNA-seq), which can detect all the active genes in an individual cell at a given time.

Looking at intermediate states

Using scRNA-seq, the researchers were able to study the entire gene expression profile of individual human embryonic stem cells throughout the differentiation protocol, which takes a total of sixty days. This allowed them to map out all the transient states within a population as they grew into retinal pigment cells, but also to optimize the protocol and suppress the growth of non-RPE cells, thus preventing the formation of contaminant cell populations. "The aim is to prevent mixed cell populations at the time of transplantation, and to make sure the cells at the endpoint are similar to original RPE cells from a patient's eye," says Lederer.

What they found was that on the way to becoming RPE cells, stem cells go through a process very similar to early embryonic development. During this, the cell culture took up a "rostral embryo patterning", the process that develops the embryo's neural tube, which will go on to become its brain and sensory systems for vision, hearing, and taste. After this patterning, the stem cells began to mature into RPE cells.

Eye-to-eye: Transplanting RPE cells in an animal model

But the point of the differentiation protocol is to generate a pure population of RPE cells that can be implanted in patients' retinas to slow down macular degeneration. So the team transplanted their population of cells that had been monitored with scRNA-seq into the subretinal space of two female New Zealand white albino rabbits, which are what scientists in the field refer to as a "large-eyed animal model". The operation was carried out following approval by the Northern Stockholm Animal Experimental Ethics Committee.

The work showed that the protocol not only produces a pure RPE cell population but that those cells can continue maturing even after they

have been transplanted in the subretinal space. "Our work shows that the differentiation protocol can develop safe and efficient pluripotent stem cell-based therapies for age-related macular degeneration," says Dr. Fredrik Lanner, who is currently making sure the protocol can be soon used in clinics.

More information: Fredrik Lanner, Molecular profiling of stem cell-derived retinal pigment epithelial cell differentiation established for clinical translation, *Stem Cell Reports* (2022). DOI: 10.1016/j.stemcr.2022.05.005

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