Small molecules vs. teratomas

By Kai-Jye Lou, Staff Writer

Researchers at **The Hebrew University of Jerusalem** and **Roche** have identified small molecules that prevented human pluripotent stem cells from forming teratomas in mice following transplantation.[1] The molecules could be useful for reducing the tumorigenicity risk of stem cell–derived cell therapies.

Cell therapies derived from stem cells can be produced by differentiating embryonic stem cells (ESCs) or induced pluripotent stem (iPS) cells into cell types unique to the diseased tissue. However, that differentiation process may also leave behind residual undifferentiated cells, which can develop into a type of tumor known as a teratoma.[2]

Thus, an ongoing challenge has been to ensure that a stem cell–derived therapy contains no teratoma-producing cells prior to transplantation. Indeed, teratoma risk has prompted the **FDA** to require developers of stem cell–derived therapies to run assays and long-term studies showing no residual undifferentiated cells and no tumor formation in animals.

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The Hebrew University and Roche researchers reasoned that a small molecule–based approach might be useful for eliminating undifferentiated cells from stem cell therapy preparations. To do that, they designed a series of screens to detect compounds that reduced the viability of undifferentiated human ESCs without being cytotoxic to differentiated cell lines.

A high throughput screen of over 52,000 small molecules identified 15 pluripotent cell–specific inhibitors—dubbed PluriSIns. In cell culture studies, these compounds were cytotoxic to both human ESCs and iPS cells but not to differentiated cells derived from these stem cells.

The researchers showed that the lead PluriSIn selectively killed undifferentiated pluripotent cells by inhibiting stearoyl-CoA desaturase-1 (SCD1), a key enzyme that mediates oleic acid biosynthesis. Moreover, oleic acid supplementation prevented PluriSIn-induced cell death in undifferentiated cells.

In mice injected with a mixture of differentiated cells and undifferentiated stem cells, none of the mice receiving cell mixtures pretreated with the lead PluriSIn developed teratomas, whereas all mice receiving vehicle-treated cell mixtures did.

Results were published in *Cell Stem Cell*. The corresponding author is Nissim Benvenisty, a professor in the Department of Genetics at The Hebrew University.

"We identified for the first time highly selective molecules that eliminate undifferentiated pluripotent stem cells in a robust and efficient manner that do not also jeopardize more differentiated cell types, including adult stem and progenitor cells," said Marcus Boehringer, a coauthor on the paper and head of external innovation and alliances in cardiovascular and metabolic diseases at Roche. "We believe this is a promising strategy for mitigating the risk of teratoma formation associated with stem cell–derived therapies."

Uri Ben-David added that the reported data also highlight the potential metabolic vulnerability of undifferentiated pluripotent cells to SCD1 and fatty acid biosynthesis. Ben-David is the paper's lead author and a graduate student in the Department of Genetics at The Hebrew University.

The result suggests other compounds that inhibit SCD1 or oleic acid biosynthesis also could have utility for the selective killing of undifferentiated cells.

"The most interesting and important result to me is that a small molecule was sufficient to selectively eliminate the teratoma-promoting cells and that genetically manipulated cells and antibodies were not required," said Micha Drukker, group leader of the Pluripotent Stem Cell Differentiation Lab and head of the Human Induced Pluripotent Stem Cell Unit at **Helmholtz Center Munich**.

"From a research publication point of view, I think this is a very promising result," said Emile Nuwaysir, VP and COO at **Cellular Dynamics International Inc.** "The results demonstrate that pluripotent cells and differentiated cells generated from pluripotent cells can have differential sensitivity to a particular chemical."

Easy and efficient

PluriSIns could potentially have fewer limitations and be more efficient and cost effective than previously described experimental methods to remove undifferentiated pluripotent cells from the final cellular product.

Those other approaches have included antibody-mediated depletion of undifferentiated cells, cell sorting and genetic modification techniques. However, those methods have shortcomings including prior knowledge of the molecular target, limited throughput and added safety concerns.

Antibody-mediated depletion of the unwanted cells requires prior knowledge of the pluripotent cell–specific surface antigen to target, whereas such knowledge was not a requirement for PluriSIns. Moreover, antibodies are generally more expensive than small molecules.

Methods based on cell sorting can damage the cells and have a direct trade-off between throughput and accuracy. Sorting-based methods also dissociate cultured cells into single units and thus would not be amenable for use in cases for which 3D culture systems and/or complex

structures of cells are desired.

Drukker and colleagues at the **Stanford University School of Medicine** previously developed a method to remove undifferentiated pluripotent cells from culture that uses antibodies targeting stage-specific embryonic antigen-5 (SSEA-5) for fluorescence-activated cell sorting (FACS).[3,4]

Genetically modifying pluripotent cells with a selectable marker that makes them easy to target and eliminate with a small molecule is another approach, but such modifications would add additional concerns to a class of therapy that already has a very high bar for safety.[5–8] Companies such as Cellular Dynamics already use genetic modification approaches to generate pure populations of research-grade cells, for which such concerns are less relevant.

Use of PluriSIns does not require genetic modification and does not involve dissociating the cells into individual units. PluriSIns thus could raise fewer safety concerns and be more broadly applicable than the previously reported methods.

"We believe PluriSIns could be easily incorporated into the process of manufacturing a pluripotent stem cell–derived therapy," Ben-David told *SciBX*. "PluriSIns can be added to culture for a limited period of time once the differentiation process has already ended and then be washed away prior to the transplantation of the cells."

"If shown to be safe for therapeutic products, I think these molecules have the potential to become a more efficient method for mitigating teratoma risk than previously reported methods based on genetic elimination and cell depletion using sorting with antibodies," added Drukker. "The small molecule approach described in this study appears to be simple and probably would not be expensive to apply."

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Exploring mechanisms and applications

The Hebrew University and Roche groups are now looking to further explore the mechanism and potential applications of the PluriSIns.

"First, we want to try to characterize the mechanism of action of additional PluriSIns," said Ben-David. "Second, we intend to examine PluriSIns' compatibility with specific clinically relevant differentiation protocols. Third, we will use mouse models to examine PluriSIn application *in vivo* in order to find out whether PluriSIns may be applied during the transplantation of the cells or even during the time course of teratoma formation."

Both Drukker and Nuwaysir added that it will be important to show that the PluriSIns can effectively eliminate undifferentiated cells without compromising the efficacy of a clinical-grade cell therapy product.

Nuwaysir noted that markers of cell death are just one indicator of toxicity and that future studies should monitor differentiated cells for other markers of toxicity as well. He added that it will be important to evaluate the efficacy of PluriSIns in mixed cell cultures that recapitulate the mixture of a batch of clinical product and in 3D culture systems because the efficacy of chemical selection processes can vary across such settings.

He also cautioned that the efficacy of chemical selection processes can vary considerably depending on the purity of the product, so it will be important to test the PluriSIns under different purity conditions as well.

The university and Roche have filed two patent applications covering the use of PluriSIns and SCD1 inhibitors to selectively eliminate human pluripotent stem cells and prevent teratoma formation. The work is available for licensing through **Yissum**, the technology transfer company of The Hebrew University, and from Roche.

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COMPANIES AND INSTITUTIONS MENTIONED

Cellular Dynamics International Inc., Madison, Wis.
Food and Drug Administration, Silver Spring, Md.
The Hebrew University of Jerusalem, Jerusalem, Israel
Helmholtz Center Munich, Munich, Germany
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Stanford University School of Medicine, Stanford, Calif.
Yissum, Jerusalem, Israel