Eliminating teratomas

By Lauren Martz, Staff Writer

A University of California, San Diego team has developed a genetic method to prevent embryonic stem cell therapies from forming cancerous teratomas following transplantation.¹ The method was safe in mice, although questions remain about whether the genetic modifications will raise safety and regulatory concerns in patients.

Teratomas are a type of tumor tissue that result from the abnormal development of pluripotent cells when transplanted *in vivo*. They are usually benign, but malignant teratomas can occur.

In human embryonic stem cell (hESC)-based therapies, pluripotent stem cells are differentiated into cells of a desired type that are then transplanted into patients. Some of the cells may not fully differentiate, which results in cell cultures containing some fraction of undifferentiated pluripotent cells that could form malignant teratomas upon transplantation.

The challenge is ensuring that a stem cell-based therapy contains only differentiated cells prior to transplantation.

Two common approaches to the problem have been cell sorting, which removes any pluripotent cells prior to transplantation, or antibody-based cytotoxic therapies that target pluripotent cells. However, cell sorting can damage the differentiated cells, and antibody-based targeting requires identification of pluripotent cell-specific surface antigens.

Now, a UCSD team led by Yang Xu, a professor of molecular biology, has developed a genetic approach to prevent teratoma formation. The approach is based on prior genetic work by other labs that used viral vectors to express an inducible suicide gene in pluripotent cells, thereby eliminating the cells and reducing the risk of teratoma formation.

In that prior work, groups at The Hebrew University of Jerusalem and Sun Yat-Sen University induced expression of herpes simplex virus thymidine kinase (HSV-tk) in hESCs using different genetic methods.^{2,3} The alteration rendered the cells sensitive to Roche's Cytovene ganciclovir, a drug for cytomegalovirus that targets HSV-tk.

Although those two methods prevented teratoma formation in mice, both used viral-based gene delivery, which itself creates the risk of random genetic insertions and mutagenesis that can lead to cancer. In addition, ganciclovir was toxic to healthy cells at the high concentrations used.

The UCSD team hypothesized that one way to avoid those problems might be to use a mutant form of the HSV-tk gene that rendered cells hypersensitive to ganciclovir and then insert that gene into a locus in the genome of the pluripotent cells using homologous recombination.

In various hESC lines, the team inserted the mutant HSV-tk gene into the 3' untranslated region of the *Nanog homeobox* (NANOG) gene,

which is specifically and highly expressed in pluripotent and partially differentiated cells but not in fully differentiated cells. The group used bacterial artificial chromosome (BAC)-based targeting for homologous recombination.

In culture with ganciclovir, mutant HSV-tk-expressing hESCs (TK-hESCs) were eliminated, whereas unmodified parental hESCs were unaffected. Without ganciclovir, TK-hESCs proliferated and differentiated as normal, suggesting the drug caused inducible hESC-specific cell death.

In immunocompromised mice, transplanted TK-hESCs or parental hESCs formed teratomas. Ganciclovir treatment beginning one day after hESC transplantation prevented teratoma formation from TK-hESCs but not from parental cells.

To more closely mimic clinical conditions, the team next transplanted partially differentiated TK-hESCs into mice and showed that ganciclovir prevented teratomas from cells derived from TK-hESCs but not from parental hESCs.

Finally, the group carried out spiking studies to determine whether treatment with ganciclovir could eliminate very small numbers of undifferentiated TK-hESCs in culture. In cultured fibroblasts, ganciclovir eliminated even a single TK-hESC.

"This work is the first step to prove that the strategy works. Next, they need to do more than prove that it can eliminate teratomas. They need to prove that it can allow differentiation of hESCs into desired therapeutic cell types that have preserved viability and function after differentiation without causing side effects to those cells," said Weidong Le, professor of neurology at Baylor College of Medicine.

"In order to prove this, they would have to test the hESC-derived cells in particular animal models of disease such as transplantation of differentiated neurons into the CNS in models of neurodegenerative disorders," continued Le. "For neurological diseases, they would also need to prove that the neurons can populate the brain tissue, that the drug they use can penetrate the brain and target the desired gene and that it can prevent the formation of teratomas."

Regulatory hurdles

Xu said his team plans to determine whether the strategy can eliminate the risk of teratomas during hESC-based therapy for human diseases such as type 1 diabetes.

In addition to confirming the efficacy of the hESC-derived cells in disease models, the team will need to perform extensive safety studies.

"This strategy uses foreign DNA and genetically modified cells, which will induce scrutiny from the regulatory agencies and will be hard to get into the clinic," said Robert Lanza, CSO of Advanced Cell Technology Inc.

"If you have an important cell type that is not able to be fully differentiated in culture, a strategy like this could be useful in eliminating the undifferentiated population. The problem still remains that there could be cell types other than undifferentiated cells that contaminate the culture."

**—Robert Lanza,
Advanced Cell Technology Inc.**

“Although this strategy improves safety by eliminating the undifferentiated cells to possibly reduce teratoma risk, it may introduce other dangers such as increasing mutagenesis.”

Xu countered that the use of gene targeting by homologous recombination should reduce or eliminate the risks associated with other strategies to genetically modify the cells, such as virus-mediated gene delivery.

“Unlike the cancer risk associated with viral vectors that are integrated into the genome randomly, the *TK* gene is introduced into one specific locus,” he said. “We will perform whole-genome sequencing to ensure that no other mutations are introduced in knock-in hESCs. Once it is confirmed that no other random integration occurs in the knock-in hESCs via whole-genome sequencing, those genetically modified hESCs will be suitable for human use.”

Advanced Cell Technology has hESC-derived retinal pigment epithelium (RPE) cells in Phase I testing to treat Stargardt’s disease and dry age-related macular degeneration (AMD). Lanza noted that RPE cells have a rigorous differentiation process that allows the formation of a fully and terminally differentiated RPE cell population from hESCs in culture. The company also has developed an assay to detect undifferentiated hESCs in culture prior to clinical use.

Lanza told *SciBX* that the newly published technology will likely require additional purification steps prior to use in humans. “What the agencies will require is a pure homogeneous population of cells,” he said. “If you have an important cell type that is not able to be fully differentiated in culture, a strategy like this could be useful in eliminating the undifferentiated population. The problem still remains that there could be cell types other

than undifferentiated cells that contaminate the culture. hESCs are capable of differentiating into any cell type, so while the proposed approach can eliminate undifferentiated cells, it does not ensure that a cell population is homogeneous.”

According to Xu, his team’s “approach addresses the issue of teratoma risk but does not help the purity of the lineage-specific differentiation. To obtain homogenous cells, the differentiated cells can be purified with cell type-specific antibodies or with knock-in hESCs with drug-resistant genes introduced into the lineage-specific locus.”

Xu told *SciBX* that the IP has not been patented and is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

Advanced Cell Technology Inc. (OTCBB:ACTC), Santa Monica, Calif.

Baylor College of Medicine, Houston, Texas

The Hebrew University of Jerusalem, Jerusalem, Israel

Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland

Sun Yat-Sen University, Guangzhou, China

University of California, San Diego, La Jolla, Calif.