Tracing cancer stem cells

By Tim Fulmer, Senior Writer

Three academic teams have independently developed techniques for *in vivo* detection of cancer stem cells in mouse solid tumors.¹⁻³ The methods could be useful as next-generation target discovery platforms and as screens for compounds that hit cancer stem cells.

Cancer stem cells (CSCs) in solid tumors were first identified in 2003 when researchers at the **University of Michigan Medical School** identified them in a mouse xenograft model of human breast cancer.⁴ The group's method relied on transplanting human tumor cells into immunodeficient animals—a biological context that is dramatically different from the cells' tumor niche in humans.

Subsequently, the same method was used by a variety of labs to identify CSCs in many solid cancers, including colon, brain, skin, and head and neck cancers.⁵

However, until now it has remained unclear whether the identified cells actually functioned as CSCs in an intact human or mouse tumor.^{6,7}

Thus, three academic teams independently set out to design cleaner approaches that directly detect CSCs in tumor tissue without the need for transplantation. The trio of methods all converged on similar strategies that combined genetic techniques and fluorescent imaging.

Each group began with a mouse model of solid cancer that was genetically altered to express a fluorescent protein in its tumor tissue.

A team led by Benjamin Simons and Cédric Blanpain engineered a mouse model of chemically induced skin cancer to express yellow fluorescent protein in basal tumor epithelial cells. Luis Parada and colleagues created a mouse model of glioma that expressed GFP in glioma cells. A team led by Hans Clevers created a mouse model of intestinal cancer that expressed GFP or one of its three derivatives in leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5; Gpr49)-positive intestinal adenoma cells.

In all cases, the mice developed cancer. Histological analysis of tumor tissue at various time points of disease progression allowed the researchers to trace the fluorescence signal as tumor cell lineages proliferated.

All three studies identified a subset of fluorescently labeled cancer cells that were proposed to be CSCs based on their ability to self-renew and give rise to other tumor cell types, including proliferating progenitor cells and terminally differentiated cells.

The study by Clevers and colleagues was published in *Science*. The other two studies were published in *Nature*.

Simons is professor of theoretical physics and the physics of

medicine at the **University of Cambridge**. Blanpain is a professor in the Interdisciplinary Research Institute at the **Free University of Brussels**. Parada is chair of the Department of Developmental Biology at **The University of Texas Southwestern Medical Center**. Clevers is professor of molecular genetics at the **Hubrecht Institute**.

Of mice and CSCs

The lineage-tracing methods used in the three papers “give very strong support to the cancer stem cell concept and are notable for avoiding the limitations of the transplantation methods, which gave rise to skepticism about the existence of cancer stem cells in solid cancers,” said Max Wicha, who published the 2003 paper that first used the transplantation method to identify breast CSCs.

He is director of the **University of Michigan Comprehensive Cancer Center** and professor of internal medicine and oncology at the University of Michigan Medical School.

“The advance here is that we can identify cancer stem cells in an intact tumor and tumor microenvironment, which could better reflect tumor biology than transplanting tumor cells into immunocompromised mice,” said Wicha.

However, he said that compared with the xenograft transplantation and cell culture methods currently used by CSC researchers, the lineage-tracing methods are more complex and perhaps not as straightforward to use. “At least in their current form, these new methods do not seem to be practical enough for use as discovery platforms and screens,” he said.

Wicha is a cofounder of **OncoMed Pharmaceuticals Inc.**, which develops therapeutic antibodies that target pathways dysregulated in CSCs. The company's lead compound, OMP-21M18, is in Phase I testing to treat non-small cell lung cancer (NSCLC) and pancreatic cancer in combination with chemotherapy. The mAb blocks delta-like 4 (DLL4), an activator of the Notch pathway in CSCs.

OncoMed's discovery and screening platform uses human tumor xenograft mice to identify CSC targets and test therapeutic mAbs.

In his own research, Wicha is combining CSC-targeting agents with antiangiogenic therapies. Earlier this year, he published data in the *Proceedings of the National Academy of Sciences* showing that antiangiogenic mAbs increase CSC proliferation in mouse models of breast cancer.⁸

Because the lineage-tracing methods allow study of intact tumors, “we may finally be able to determine what role cancer stem cells play in the initial events of tumor formation and how those cells drive tumor progression,” said Mick Bhatia. “That information could reveal periods of tumor progression when cancer stem cells are more susceptible to therapy.”

Bhatia is professor of biochemistry and biomedical sciences at **McMaster University** and director of the **Stem Cell and Cancer Research Institute at McMaster University**. He cofounded **Actium Research Inc.**, which has licensed a human cancer stem cell discovery platform from McMaster. The platform is based on a human pluripotent stem cell line that has CSC properties including self-

renewal and loss of differentiation capacity.⁹

Earlier this year, Bhatia and colleagues published in *Cell* that the platform had identified the small molecule dopamine receptor antagonist thioridazine as a CSC-targeting molecule.¹⁰

Verastem Inc., another company in the CSC space, also uses a cell culture platform as part of its screens of compounds that block proliferation of CSCs.¹¹ The platform is based on an immortalized human breast epithelial cell line that is genetically modified to undergo the epithelial-mesenchymal transition (EMT), a process that imparts self-renewal capability similar to that of CSCs.

In 2009, Verastem cofounder Robert Weinberg published in *Cell* that the screen identified the antibiotic salinomycin as an inhibitor of breast CSCs.¹² In 2012, the company presented additional data at the **American Association for Cancer Research** meeting in Chicago showing that salinomycin inhibited wingless-type MMTV integration site (WNT) family member signaling, a pathway known to be dysregulated in CSCs. However, the company announced earlier this year that it has put development of salinomycin on hold and is seeking to discover next-generation WNT inhibitors.

Verastem also has a preclinical program targeting focal adhesion kinase (FAK) to block growth of breast CSCs.

Weinberg is a member of the **Whitehead Institute for Biomedical Research** and professor of biology at the **Massachusetts Institute of Technology**. He did not respond to requests for an interview.

Moving forward

The corresponding authors on the papers told *SciBX* they have ideas about how to use the lineage-tracing methods in both the target discovery and compound screening setting.

"We plan to purify the cancer stem cells from the mice and use deep sequencing to generate mutational and epigenetic profiles that can be compared with the profiles of noncancer stem cells from the same mice," said Parada, corresponding author on the glioma study. "Differences between the two profiles could point to pathways that are dysregulated in cancer stem cells and might be good targets."

"We are also setting up a high throughput platform that pools tumors from multiple mice used in the studies. We will then use those tumor cells to screen small molecules and siRNA for their ability to arrest cellular metabolic activity. Hits from that screen will then be validated in primary human tumor cells, where mechanistic details can be worked out," he added.

"Because our screen is based on cancer stem cells isolated directly from the tumor in its natural environment, we believe the compounds we identify may have a better chance of therapeutic success than compounds identified using screens based on tumor transplantation or cell lines modified to have cancer stem cell-like properties," said Parada.

Simons, corresponding author on the skin cancer study, told *SciBX* his group plans to use the lineage-tracing method "to investigate the biomolecular pathways that lead to the dysregulation of the stem cell compartment."

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—Mick Bhatia,
McMaster University

He added that the method may provide a new way "to define the mode of tumor growth in different types of cancer and during metastasis and relapse, which may have important implications for the development of new therapeutic strategies."

Clevers, corresponding author on the intestinal cancer study, did not respond to interview requests.

Longer-term potential

Lineage tracing and xenograft models could potentially be combined into a single screening platform for CSCs, said Wicha.

"One method will tell us how a compound acts on transplanted human cancer stem cells,

while the other method tells us how a compound acts on cancer stem cells in an intact mouse tumor. Presumably a compound that is active in both scenarios would be prioritized as a strong development candidate," he said.

Even by itself, the lineage-tracing method could provide information on CSC biology not available from the xenograft studies. "It should be possible to modify the method used in the papers to include two color tracers, one expressed in cancer stem cells, the other in stromal stem cells. Tracing the interactions between the two over time could then show how the tumor niche and microenvironment influence the function of cancer stem cells," said Bhatia.

The CSCs identified by lineage tracing could also be used in target discovery, providing a more definitive gene and protein expression profile useful for identifying new targets specifically expressed in those cells, said Christopher Reyes, cofounder and CSO of **Eclipse Therapeutics Inc.**

Eclipse uses its CSC Rx Discovery platform to identify mAb therapeutics that inhibit growth of CSCs. Eclipse's lead compound is ET101, a preclinical mAb against an undisclosed CSC target on solid tumors.

The findings in all three papers are not covered by patents.

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COMPANIES AND INSTITUTIONS MENTIONED

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