Journal of Molecular Cell Biology Advance Access published April 16, 2014
doi:10.1093/jmcb/mju012

Letter to the Editor

Elimination of undifferentiated cancer cells by pluripotent stem cell inhibitors

Dear Editor,

According to the cancer stem cell (CSC) hypothesis, cancer cells are organized in a hierarchical manner (Reya et al., 2001). Indeed, the differentiation state of cancer cells has been linked to their proliferative potential, drug response, and ability to metastasize (Clevers, 2011). Targeting self-renewing, undifferentiated cancer cells could therefore be of importance for the success of cancer therapies. A classical case of a tumor composed of undifferentiated and differentiated cells is the tumor induced by pluripotent stem cells (PSCs) (Ben-David and Benvenisty, 2011). We have recently identified several small molecules termed pluripotent-specific inhibitors (PluriSIns) that selectively target undifferentiated tumorigenic PSCs (Ben-David et al., 2013). Several of these compounds, including PluriSIn#1, inhibit the activity of stearoyl-coA desaturase (SCD1) (Ben-David et al., 2013). SCD1 is an endoplasmic reticulum (ER)-membrane protein that plays a key role in the biosynthesis of the mono-unsaturated fatty acid oleate. This novel metabolic vulnerability is in line with the unique metabolome described in PSCs (Panopoulos et al., 2012).

Inhibition of SCD1 was previously suggested as a potential cancer therapy (Hess et al., 2010; Minville-Walz et al., 2010; Roongta et al., 2011); however, why some cancer cells are highly sensitive to SCD1 inhibition, whereas others are completely resistant to it, remains unknown. As undifferentiated human PSCs were highly sensitive to SCD1 inhibition and rapidly lost this sensitivity upon differentiation (Ben-David et al., 2013), and the processes of pluripotency induction and oncogenic transformation share many characteristics (Ben-David and Benvenisty, 2011), we speculated that the sensitivity of some cancer cells to SCD1 inhibition might be related to their differentiation state as well.

To test this hypothesis, we took advantage of a recently developed system of *in vitro* transformed CSC-like cells (Scaffidi and Misteli, 2011). When forced to stably express hTERT, H-RasV12 and SV40 LT and ST antigens, human primary skin fibroblasts undergo an oncogenic reprogramming process and acquire long-term self-renewal ability and differentiation capacity, in contrast to their hTERT-immortalized control counterparts (Scaffidi and Misteli, 2011). We therefore tested the response of these isogenic cell lines to PluriSIn#1. Interestingly, while the immortalized fibroblasts exhibited a very weak response, the transformed undifferentiated cells were extremely sensitive to PluriSIn#1, and massive cell death was observed in culture at 72 h post exposure (Figure 1A). To examine whether the direct cause of death following PluriSIn#1 exposure was indeed SCD1 inhibition, we exposed the cells to another specific inhibitor of SCD1, A939572, and obtained a highly similar differential response (Supplementary Figure S1A). Moreover, exogenous supplementation of oleate, the enzymatic product of SCD1 activity, completely rescued the cell death induced by PluriSIn#1 (Figure 1B). We repeated the experiment with a second isogenic pair of immortalized and transformed cell lines (Supplementary Figure S1B), ruling out the possibility that a cell line-specific difference unrelated to the cellular transformation underlies the differential response.

We next set out to determine whether the sensitivity of the transformed cells to SCD1 inhibition is related to their undifferentiated state. The transformed cells were differentiated into adipocytes (Scaffidi and Misteli, 2011), and were exposed to Pluri SIn#1. We found that 6 days of adipogenic differentiation were sufficient to significantly reduce the sensitivity of the transformed cells to SCD1 inhibition (Figure 1C and D). To further examine the relationship between differentiation state and oleate dependency in cancer cells, we tested a cell line of undifferentiated stem-like glioma cells (SLGCs) (Campos et al., 2010). These cells grow as neurospheres in suspension, and efficiently differentiate when cultured in adhesion in the presence of retinoic acid (RA) (Campos et al., 2010). Consistent with the findings from the transformed cell lines, undifferentiated SLGCs were highly sensitive to PluriSIn#1, but 1 week of RA-induced differentiation rendered them completely resistant to the molecule (Figure 1E and F). To examine whether it is indeed differentiation, rather than cell cycle exit, that protects cells from SCD1 inhibitor-mediated death, human PSCs were cultured in a bFGF-free medium and exposed to PluriSIn#1 prior to their actual differentiation; the sensitivity to SCD1 inhibition was not reduced in the growth factor-depleted medium (Supplementary Figure S2), suggesting that cell cycle exit is not sufficient to protect the cells.

Lastly, we examined whether undifferentiated cancer cells could be depleted from a heterogeneous population by inhibition of oleate synthesis. Transformed fibroblasts were treated with either 20 μM PluriSIn#1 or 0.02% DMSO as control for 72 h, in the presence of immortalized fibroblasts. Cells were then harvested and injected subcutaneously into immune-deficient mice ($n = 4$). Each mouse was injected with same number of control-treated cells into one side of the body and PluriSIn#1-treated cells into the other side. Six weeks later, mice were sacrificed, and tumors were measured and analyzed. PluriSIn#1-treated cells gave rise to much smaller tumors compared with control-

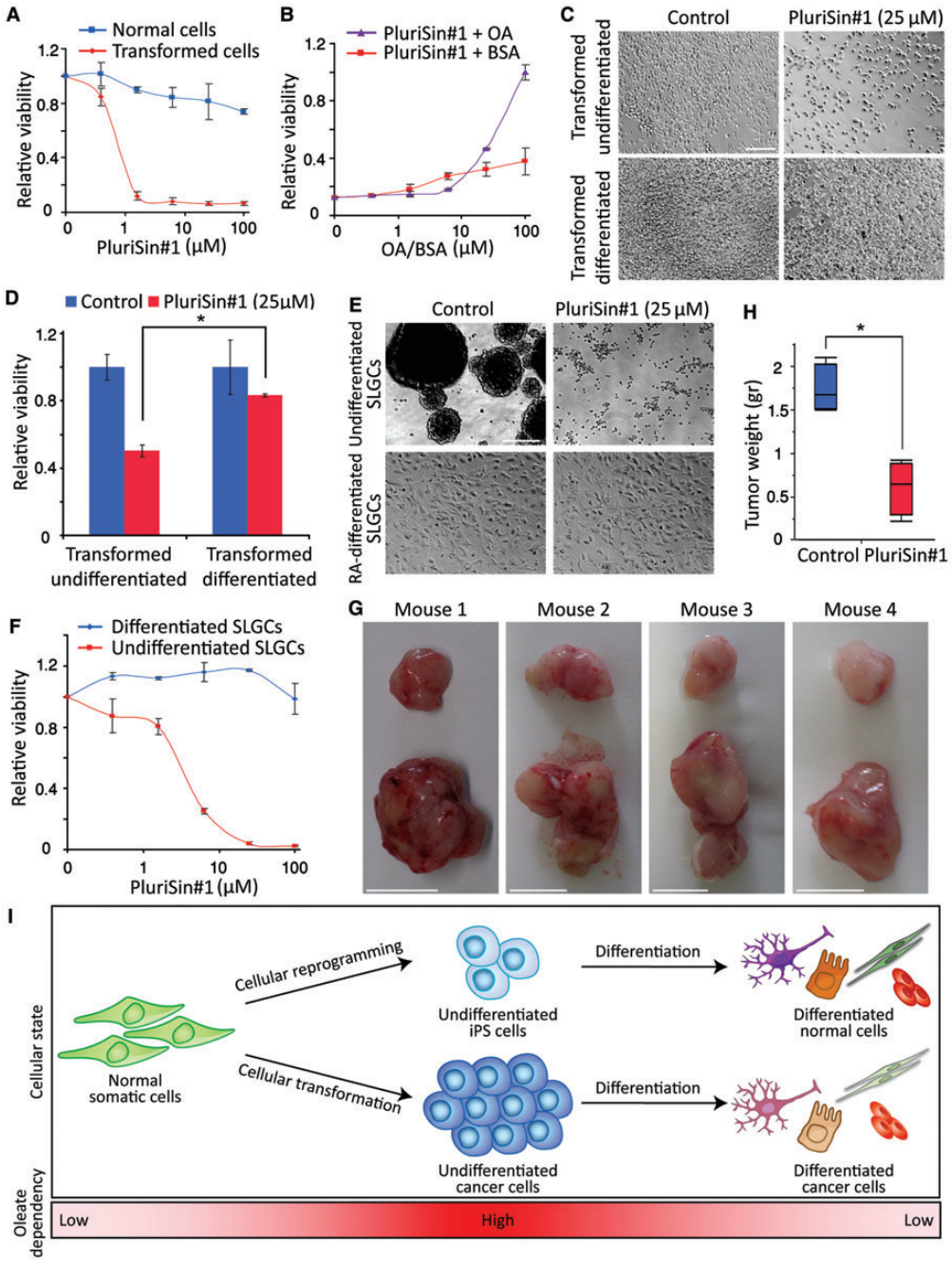

Figure 1 Cellular transformation renders undifferentiated cancer cells sensitive to SCD1 inhibition, and this sensitivity is quickly lost upon differentiation. (**A**) Quantitated cellular viability of immortalized fibroblasts (blue) and undifferentiated transformed fibroblasts (red) exposed to PluriSIn#1 for 72 h in low (2%) serum culture medium. (**B**) Quantitated cellular viability of undifferentiated transformed cells exposed to PluriSIn#1 (25 μM) for 72 h in low (2%) serum medium, in the presence of oleic-acid conjugated to BSA (purple) or BSA alone (red). (**C**) Representative images of undifferentiated transformed cells (top panel) and transformed cells after 6 days of adipogenic differentiation (bottom panel), following a 72 h exposure to control conditions (left) or PluriSIn#1 (25 μM, right). Scale bar, 100 μm. (**D**) Quantitated cellular viability of undifferentiated transformed cells (left) and differentiated transformed adipocytes (right) exposed to PluriSIn#1 (25 μM) for 72 h in adipogenic maintenance medium. The undifferentiated cells were significantly more sensitive than their differentiated counterparts, $^*P = 0.003$. (**E**) Representative images of undifferentiated SLGCs (top panel) and SLGCs after 7 days of retinoic acid (RA) differentiation (bottom panel), following a 72 h exposure to control conditions (left) or PluriSin#1 (25 μM, right). Scale bar, 200 μm. (**F**) Quantitated cellular viability of undifferentiated SLGCs (red) and RA-differentiated SLGCs (blue) exposed to PluriSIn#1 for 72 h. (**G**) Images of the tumors that were generated by transplanting the same number of a mixture of transformed and immortalized BJ-fibroblasts following a 72 h exposure to control conditions (top panel) or PluriSIn#1 (20 μM, bottom panel). Scale bar, 1 cm. (**H**) Box-plot quantification of the abovementioned tumors' weight. $^*P = 0.001$. (**I**) According to the suggested model, somatic cells acquire dependency on oleate synthesis during cellular reprogramming and cellular transformation, and lose this dependency upon differentiation. Data shown throughout the Figure are represented as means ± SD. One-tailed Student's *t*-test was used.

treated cells (Figure 1G and H). Treatment with A939572 similarly reduced the size of the generated tumors ($n = 3$, Supplementary Figure S1C). These data indicate that SCD1 inhibition significantly decreases the tumorigenicity of the heterogeneous cell population.

We have previously shown that fibroblasts resistant to SCD1 inhibition acquire the metabolic sensitivity when reprogrammed into induced PSCs, but this sensitivity is quickly lost upon differentiation (Ben-David et al., 2013). Here we demonstrate that these fibroblasts acquire the same metabolic vulnerability when they are transformed into undifferentiated stem cell-like cancer cells, but the resistance to SCD1 inhibition is conferred again once cancer cells are induced to differentiate (Figure 1I). These findings link, for the first time, the differentiation state of cancer cells with their response to inhibition of oleate synthesis, suggesting that SCD1 inhibition might be an especially effective cancer therapy for undifferentiated tumor types. This work also demonstrates that research on cellular reprogramming can yield insights into cancer biology, and validates that *in vitro* generated CSCs can be successfully used for screening anticancer drugs.

[Supplementary material is available at Journal of Molecular Cell Biology online. We thank members of the Stem Cell Unit at the Hebrew University, and especially Tamar Golan-Lev for her assistance with the graphic design of the Figures, Ofra Yanuka for her help with SLGC culturing, and Ido Sagi and Daniel Ronen for critically reading the manuscript. N.B. is the Herbert Cohn Chair in Cancer Research. U.B.-D. and A.B. are Clore Fellows. This work was supported by a Hoffmann La Roche-Yissum Collaboration grant, a DKFZ-Israel Collaboration grant (#CA-146) from the Israel Ministry of Science, and the Intramural Research Program of the National Institutes of Health (NIH), NCI, Center for Cancer Research.]

Uri Ben-David[1,2,†], Alva Biran[2,†], Paola Scaffidi[3,4,5], Christel Herold-Mende[6], Markus Boehringer[7], Eran Meshorer[2,*], and Nissim Benvenisty[1,2,*]

[1]Stem Cell Unit, Silberman Institute of Life Sciences, The Hebrew University, Jerusalem 91904, Israel
[2]Department of Genetics, Silberman Institute of Life Sciences, The Hebrew University, Jerusalem 91904, Israel
[3]National Cancer Institute, NIH, Bethesda, MD 20892, USA
[4]UCL Cancer Institute, University College London, London WC1E 6BT, UK
[5]Cancer Research UK London Research Institute, London WC2A 3LY, UK
[6]Division of Neurosurgical Research, Department of Neurosurgery, University of Heidelberg, Heidelberg, Germany
[7]F. Hoffmann-La Roche AG, Basel 4070, Switzerland
[†]These authors contributed equally to this work.
[*]Correspondence to: Eran Meshorer, E-mail: meshorer@huji.ac.il; Nissim Benvenisty, E-mail: nissimb@cc.huji.ac.il

References

Ben-David, U., and Benvenisty, N. (2011). The tumorigenicity of human embryonic and induced pluripotent stem cells. Nat. Rev. Cancer *11*, 268–277.

Ben-David, U., Gan, Q.F., Golan-Lev, T., et al. (2013). Selective elimination of human pluripotent stem cells by an oleate synthesis inhibitor discovered in a high-throughput screen. Cell Stem Cell *12*, 167–179.

Campos, B., Wan, F., Farhadi, M., et al. (2010). Differentiation therapy exerts antitumor effects on stem-like glioma cells. Clin. Cancer Res. *16*, 2715–2728.

Clevers, H. (2011). The cancer stem cell: premises, promises and challenges. Nat. Med. *17*, 313–319.

Hess, D., Chisholm, J.W., and Igal, R.A. (2010). Inhibition of stearoylCoA desaturase activity blocks cell cycle progression and induces programmed cell death in lung cancer cells. PLoS One *5*, e11394.

Minville-Walz, M., Pierre, A.S., Pichon, L., et al. (2010). Inhibition of stearoyl-CoA desaturase 1 expression induces CHOP-dependent cell death in human cancer cells. PLoS One *5*, e14363.

Panopoulos, A.D., Yanes, O., Ruiz, S., et al. (2012). The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming. Cell Res. *22*, 168–177.

Reya, T., Morrison, S.J., Clarke, M.F., et al. (2001). Stem cells, cancer, and cancer stem cells. Nature *414*, 105–111.

Roongta, U.V., Pabalan, J.G., Wang, X., et al. (2011). Cancer cell dependence on unsaturated fatty acids implicates stearoyl-CoA desaturase as a target for cancer therapy. Mol. Cancer Res. *9*, 1551–1561.

Scaffidi, P., and Misteli, T. (2011). In vitro generation of human cells with cancer stem cell properties. Nat. Cell Biol. *13*, 1051–1061.