Cell PRESS
Cell Stem Cell
Previews

Do Cells *let-7* Determine Stemness?

Peter Dröge[1,*] and Curt A. Davey[2]
[1]Division of Genomics and Genetics
[2]Division of Structural and Computational Biology
School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551
*Correspondence: pdroge@ntu.edu.sg
DOI 10.1016/j.stem.2007.12.003

During oncogenic transformation, microRNA levels of the translation-regulatory factor *let-7* correlate inversely with expression of the HMGA2 oncoprotein. In a recent issue of *Cell*, Yu et al. (2007) now provide evidence that the *let-7*/HMGA2 linkage could be a signature of cancer stem cells in vivo, with broader implications for stem cell research.

The physiological significance of the strong link between the *let-7* family of tumor-suppressor mircoRNAs (miRNAs) and the high-mobility group AT-hook 2 (HMGA2) oncoprotein during oncogenic transformation has remained unclear, because researchers have almost exclusively investigated established human cancer cell lines. Song, Lieberman, and colleagues (Yu et al., 2007) now provide compelling evidence that the *let-7*/HMGA2 connection is indeed of clinical importance. Interestingly, it emerged that this linkage might represent a functional signature of elusive cancer stem cells (CSCs), referred to as tumor-inducing cells by Yu et al. (2007), for at least some types of cancer. These findings reach beyond tumorigenesis and likely impinge on embryonic stem cell (ESC) biology.

According to the cancer stem cell hypothesis, CSCs are rare, slow-dividing, and self-renewing cells. Isolated breast CSCs exhibit a $CD44^{+}CD24^{-/low}$ phenotype and can grow into so-called mammospheres in suspension culture, which is a measure of self-renewal capability. Based on these criteria, Yu et al. (2007) took advantage of evidence that chemotherapy-resistant CSCs might reside in tumors of breast cancer patients (Al-Hajj, 2007) and enriched these cells before and after chemotherapy. Chemotherapy increased the fraction of CSCs in tumor specimens by about 14-fold, which is, of course, an unsettling finding considering current cancer treatment regimens. This selection strategy was subsequently applied to enrich CSCs in the established hypertriploid human breast adenocarcinoma cell line SKBR3. After injecting SKBR3 cells into chemotherapy-treated, immunocompromised mice, and subsequent to two serial in vivo passages of xenografts, a progenitor cell line termed SK-3rd was generated that contained ~16% CSCs.

Yu et al. (2007) established that the mammosphere-derived SK-3rd cells exhibited self-renewing and in vitro differentiating capability, with a significant increase in tumor formation and metastasis. Also, western blot analysis demonstrated that cells expressed ESC marker OCT4, which was downregulated during induced in vitro differentiation. Taken together, this shows that CSCs display features attributed to multipotent stem cells. It would have been interesting, however, had the authors also obtained information on the expression of other prominent ESC markers, such as SOX2, NANOG, or UTF1, which could provide insight into the degree of stemness of CSCs.

Yu et al. (2007) demonstrated that among 52 miRNAs analyzed in mammosphere-derived SK-3rd cells, the most significantly reduced expression was for the *let-7* family. In addition to HMGA2, RAS is also a prominent *let-7* target, and the expression of both oncoproteins, which is high in SK-3rd and greatly reduced in differentiated tumor cells, was inversely correlated with that of *let-7*. Importantly, the authors then turned their attention back to CSCs enriched directly from clinical specimens and showed that *let-7* expression was also markedly reduced when compared with normal breast or nonselected tumor cells. Obviously, this is a very important result that strengthened the clinical significance of their findings obtained with sphere-derived SK-3rd cells. Notably, others have also recently reported an inverse in vivo correlation between *let-7* and HMGA2 in primary tumors derived from 100 patients diagnosed with ovarian cancer (Shell et al., 2007; Park et al., 2007).

Yu et al. (2007) then confirmed the functional role of the *let-7* signature of CSCs by demonstrating that self-renewal and maintenance of an undifferentiated state required reduced *let-7* levels. Intriguingly, inhibition of *let-7* activity in SKBR3 cells through antisense oligonucleotides (ASOs) enhanced their self-renewal capability, thereby indicating some degree of induced dedifferentiation. Furthermore, *let-7* targeting of RAS appeared to control self-renewal of CSCs, whereas HMGA2 mediated maintenance of an undifferentiated state. The latter nuclear factor is also involved in cell proliferation control because a knockdown of HMGA2 in differentiating SK-3rd cells reduced proliferation rates.

A very interesting picture now emerges in which the *let-7*/HMGA2 linkage in CSCs described by Yu et al. (2007) is strikingly similar to that in ESCs (Figure 1). *let-7* levels in ESCs are also greatly reduced, increase with the level of differentiation, and are inversely correlated with the expression of HMGA2 (Lee et al., 2005; Li et al., 2006). The finding that reduced *let-7* levels control self-renewal and differentiation in CSCs through RAS and HMGA2, respectively, may hint at similar functional roles in ESCs. In fact, in human and mouse ESCs, HMGA2 is known to play a role in differentiation control and cell proliferation (Li et al., 2007). It is almost certain that other *let-7* targets are involved, but the pygmy phenotype of *HMGA2* knockout mice with significantly reduced fat tissue (Zhou et al., 1995) confirms a central role for the *let-7*/HMGA2 connection in this scenario.

Another indication that this connection is of functional significance in ESCs

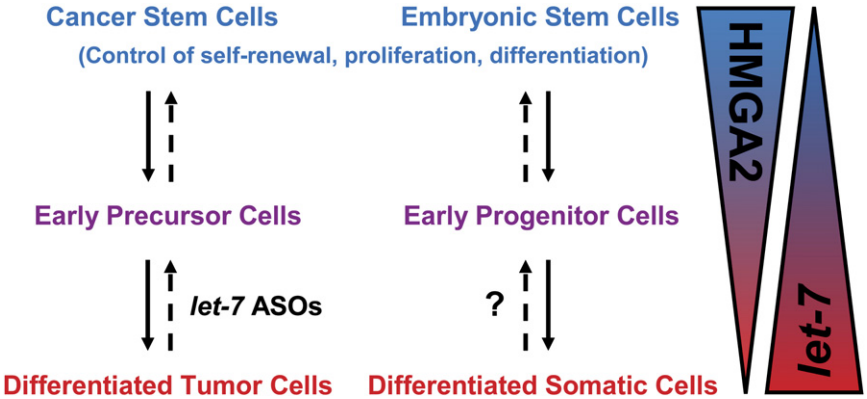

Figure 1. The Inverse Correlation between *let-7* and HMGA2 Expression in Cancer Stem Cells and Embryonic Stem Cells

See text for details. *let-7* ASOs refer to antisense oligonucleotides that target the *let-7* family of miRNAs and induce dedifferentiation of tumor cells. This is possible because of the high degree of sequence conservation within this family. Whether a similar effect can be achieved with differentiated somatic cells is not known.

comes from a recent study by Weedon et al. (2007), who described an association between increased human height and a particular single nucleotide polymorphism (SNP) in the *HMGA2* gene—rs1042725. Upon closer inspection, we find that rs1042725 maps proximal to the third functional binding site for *let-7* in the 3′ untranslated region (3′UTR) of HMGA2 mRNA (see Shell et al., 2007 for an analysis of *let-7* binding sites in the 3′UTR). This SNP is located ten nucleotides downstream of the *let-7* seed sequence. It is therefore tempting to suggest that HMGA2 expression in the individuals identified with increased height may be augmented during early embryonic development as a result of decreased association of *let-7* with this 3′UTR translation-regulatory element. Based on HMGA2's known role in differentiation control and stem cell proliferation, elevated levels could then affect the population size of stem or early progenitor cells.

The growing evidence for an important functional role of reduced *let-7* levels both in ESCs and CSCs raises an interesting question. Is there a causative relationship between the control of *let-7* expression and stemness? Yu et al. (2007) provide the first glimpse of an answer by showing that ASOs targeting *let-7* triggered dedifferentiation of SKBR3 cells (see Figure 1). Based on this finding, one might now be tempted to explore *let-7* ASOs as a component of strategies for transcription factor-induced pluripotency in human somatic cells (Takahashi et al., 2007). This is, of course, but one of many pressing questions regarding the *let-7*/HMGA2 relationship to stemness.

REFERENCES

Al-Hajj, M. (2007). Curr. Opin. Oncol. *19*, 61–64.

Lee, Y.S., Kim, H.K., Chung, S., Kim, K.-S., and Dutta, A. (2005). J. Biol. Chem. *280*, 16635–16641.

Li, O., Vasudevan, D., Davey, C.A., and Dröge, P. (2006). Genesis *44*, 523–529.

Li, O., Li, J., and Dröge, P. (2007). FEBS Lett. *581*, 3533–3537.

Park, S.-M., Shell, S., Radjabi, A.R., Schickel, R., Feig, C., Boyerinas, B., Dinulesco, D.M., Lengyel, E., and Peter, M.E. (2007). Cell Cycle *6*, 2585–2590.

Shell, S., Park, S.-M., Radjabi, A.R., Schickel, R., Kistner, E.O., Jewell, D.A., Feig, C., Lengyel, E., and Peter, M.E. (2007). Proc. Natl. Acad. Sci. USA *104*, 11400–11405.

Takahashi, K., Tanabe, K., Ohnuki, M., Natita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Cell *131*, 861–872.

Weedon, M.N., Lettre, G., Freathy, R.M., Lindgren, C.M., Voight, B.F., Perry, J.R.B., Elliot, K.S., Hackett, R., Guiducci, C., Shields, B., et al. (2007). Nat. Genet. *39*, 1245–1250.

Yu, F., Yao, H., Zhu, P., Zhang, X., Pan, Q., Gong, C., Huang, Y., Hu, X., Su, F., Lieberman, J., and Song, E. (2007). Cell *131*, 1109–1123.

Zhou, X., Benson, K.F., Ashar, H.R., and Chada, K. (1995). Nature *376*, 771–774.

Note Added in Proof

A related paper showing that *let-7* expression is also reduced in the primitive fraction of a mouse mammary epithelial cell line was published after this article was completed (Ibarra, I., Erlich, Y., Muthuswamy, S.K., Sachidanandam, R., and Hannon, G.J. [2008]. A role for microRNAs in maintenance of mouse mammary epithelial progenitor cells. Genes Dev. *21*, 3238–3243).