

VIEWPOINT

MicroRNAs: shortcuts in dealing with molecular complexity?

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Abstract

Recent studies from Clarke's group published in the journal *Cell* indicate that miRNAs may be the elusive universal stem cell markers that the field of cancer stem cell biology has been seeking. Distinct profiles of miRNAs appear to reflect the state of cell differentiation not only in breast cancer cells, but also in normal mammary epithelial cells. Moreover, they are conserved across tissues and species. The authors of this work also show evidence that downregulation of miRNA-200c in normal and malignant breast stem cells and in embryonal carcinoma cells has functional relevance, being responsible for the proliferative potential of these cells *in vitro* and *in vivo*.

MicroRNAs are able to control complex programs by regulating the expression of hundreds of genes simultaneously. Since their discovery almost three decades ago, numerous alterations in miRNA expression with varying underlying mechanisms were associated with human malignancies [1]. The study by Shimono and colleagues now shows that certain miRNAs may control the molecular makeup of stemness, and may be a shared trait of stem cells from various origins: embryonal and adult stem cells, normal and malignant stem cells [2]. This molecular similarity between normal and malignant stem cells re-enforces the concept put forward by the cancer stem cell model, according to which stem cells and early progenitor cells are more susceptible to transformation than their differentiated counterparts [3]. This may be due in part to a molecular intracellular context that sustains self-renewal and/or high proliferative potential.

Shimono and colleagues performed a comparative analysis of purified CD44⁺CD24⁻lin⁻ cancer stem cell populations from three different breast cancers, which

revealed differential expression of 37 miRNAs [2]. Among these, three clusters of miRNAs were consistently downregulated in an additional eight breast cancer samples: miRNA-183-96-182, miRNA-200c-141 and miRNA-200b-200a-429. The latter two clusters have the same seed sequence, suggesting that they may have overlapping targets. Remarkably, this downregulation appeared to be conserved in embryonal carcinoma cells (Tera-2 cells), in normal and malignant mammary stem cells of mouse origin defined by the CD24⁻CD49f⁺lin⁻ phenotype [4], and in normal mammary stem/progenitor cells defined by the CD49f⁺EpCAM^{neg/low}CD31⁻CD45⁻ phenotype [5]. When miRNA-200c levels were restored in any of these cells, they lost the ability to proliferate *in vitro*, as demonstrated by a dramatic decline in clonogenicity, and they lost the ability to proliferate *in vivo*, as demonstrated by an inability to generate tumors or normal outgrowths upon orthotopic implantation in mice.

In a long list of genes potentially regulated by miRNA-200c, the authors focused on BMI-1 for further validation, because of its recognized role in self-renewal. Bmi-1 is a polycomb group protein that, in a variety of experimental systems, appeared to be necessary for self-renewal and proliferation of stem cells and appeared able to repress differentiation, senescence and apoptosis. Impressively, BMI-1 expression restored the clonogenicity of MMTV-Wnt 1 breast cancer cells expressing miRNA-200c. The MMTV-Wnt 1 cell line was used in the study as an experimental model of mouse tumors with an expanded stem cell population [4]. Expression of miRNA-200c in these cells dramatically reduced clonogenicity, which was restored to levels seen in uninfected cells by lentiviral-driven expression of Bmi-1.

The implications of these findings are several-fold. First, these results suggest the potential use of miRNAs as stem cell markers. Fairly simple phenotypes have so far been used as stem cell markers, defined by the presence of a maximum of 10 to 12 antigens or by the presence of a particular cell function, such as transmembrane efflux (SP population) [6] or enzymatic activity (aldehyde dehydrogenase) [7]. Since miRNAs are regulators of large molecular programs, they define much more complex phenotypes. Moreover, they appear to confer specific

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developmental identities to cells. It would be very interesting to see whether the upregulation of the miRNA clusters miRNA-214, miRNA-127, miRNA-142-3p and miRNA-199a, identified in the same study, is involved in promoting stem-cell-specific functions, such as self-renewal and maintenance of an undifferentiated state.

Another potential implication is developing cancer therapies by targeting miRNAs, as discussed in the commentary that accompanied Shimono and colleagues' paper [8]. Conceptually identical with cancer therapy through differentiation, miRNA targeting puts a molecular face to this old notion. By changing the intracellular molecular context, by interfering with the cells' stemness, we may be able to annihilate the consequences of cancer-initiating and cancer-promoting events without directly targeting them. If clusters of miRNAs with key roles in this cell-fate determination are identified, it may be possible to circumvent the challenging task of elucidating networks of molecular interactions responsible for cell-fate determination and the complexity related to redundancy, feedback regulatory and compensatory mechanisms.

What would be the caveats of such approaches? The same characteristics that make miRNA appealing targets may represent important limitations. As the authors of this study mention, the number of miRNAs targets is typically large. Moreover, it includes genes that encode for molecules with opposing functions. For example, the TargetScan analysis of miRNA-200c indicates about 800 possible targets – some of them, such as Bmi-1, Notch1 and SOX2, whose upregulation was associated with self-renewal; and other targets, such as PTEN, whose downregulation was associated with an undifferentiated state and self-renewal [9,10]. This is consistent with previous observations that both oncogenes and tumor suppressors, both genes promoting and suppressing cell proliferation, and both proapoptotic and antiapoptotic genes can be targets of a certain miRNA [2]. From this perspective, the large number of targets may not be advantageous when developing miRNA-targeted strategies.

In conclusion, elucidating the role of miRNAs in cell-fate determination would be an important step for understanding the basic biology of stem cells and their role during malignant transformation and tumor

progression. Important applications may be developed based on this knowledge, such as using miRNAs as stem cell markers. Targeting miRNA also emerges as an opportunistic shortcut to circumvent the complexity resulting from feedback regulatory and compensatory mechanisms when aiming to effectively change cellular programs that dictate cell fate. Developing therapeutic approaches based on this concept should be considered with extreme caution, however, given the considerable potential for side effects.

Abbreviations

miRNA = microRNA.

Competing interests

The authors declare that they have no competing interests.

Published: 19 January 2010

References

1. Bartel DP: **MicroRNAs: target recognition and regulatory functions.** *Cell* 2009, **136**:215-233.
2. Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, Diehn M, Liu H, Panula SP, Chiao E, Dirbas FM, Somlo G, Reijo Pera RA, Lao K, Clarke MF: **Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells.** *Cell* 2009, **138**:592-603.
3. Liu S, Dontu G, Wicha MS: **Mammary stem cells, self-renewal pathways, and carcinogenesis.** *Breast Cancer Res* 2005, **7**:86-95.
4. Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE: **Generation of a functional mammary gland from a single stem cell.** *Nature* 2006, **439**:84-88.
5. Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li H, Eaves CJ: **Purification and unique properties of mammary epithelial stem cells.** *Nature* 2006, **439**:993-997.
6. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC: **Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo.** *J Exp Med* 1996, **183**:1797-1806.
7. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu SL, Schott A, Hayes D, Birnbaum D, Wicha M, Dontu G: **ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome.** *Cell Stem Cell* 2007, **1**:555-567.
8. Dirks PB: **MicroRNAs and parallel stem cell lives.** *Cell* 2009, **138**:423-424.
9. Wang J, Rao S, Chu J, Shen X, Levasseur DN, Theunissen TW, Orkin SH: **A protein interaction network for pluripotency of embryonic stem cells.** *Nature* 2006, **444**:364-368.
10. Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, Morrison SJ: **Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells.** *Nature* 2006, **44**:475-482.

doi:10.1186/bcr2455

Cite this article as: Dontu G, de Rinaldis E: **MicroRNAs: shortcuts in dealing with molecular complexity?** *Breast Cancer Research* 2010, **12**:301.