a transient period (8–12 days: Brambrink et al., 2008; Stadtfeld et al., 2008). Together, these findings suggest that nonintegrating reprogramming methods may be developed to provide transient overexpression of the transcription factors, which will positively impact the ability to translate iPS technology into therapies.

To begin to determine the origin of the cell that gives rise to iPS colonies, Aoi et al. (2008) used a lineage-tracing strategy that identifies cells that, at some time, have expressed the hepatic gene albumin. Their results indicate that liver-derived iPS cells were almost all positive for this reporter, suggesting that lineage-committed cells can be reprogrammed to an ES-like state. However, although albumin is expressed in mature hepatocytes and liver progenitors, it could conceivably have been activated during the reprogramming process in vitro. To conclusively demonstrate that iPS cells arise from terminally differentiated cells and not rare stem cells, populations with differentiation-associated genomic rearrangements, such as lymphocytes, will need to be examined.

Although the hypothesis that iPS cells arise from a rare stem cell remains possible until an unambiguously genetically marked cell can be reprogrammed to indicate the differentiation state of the donor cell, Yamanaka's latest studies suggest that the low efficiency of reprogramming is not a result of directed insertional mutagenesis and that factor-induced reprogramming is a universal process that is not restricted to particular cell types. Given that the overall efficiency of reverting early reprogramming intermediates into iPS cells is still low (Brambrink et al., 2008; Stadtfeld et al., 2008), transcription factor-induced reprogramming must require rare stochastic, likely epigenetic, events. Analyzing subpopulations of iPS intermediates from multiple tissues via genome-wide approaches for factor binding and chromatin changes should reveal important molecular events that occur during this cascade. Such insights may lead to safer, more efficient reprogramming methods that will be necessary to translate iPS cells into therapeutic tools.

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Cancer: Inappropriate Expression of Stem Cell Programs?

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Cancer stem cells (CSCs) are a subpopulation of cancer cells that possess characteristics, including self-renewal, associated with normal stem cells. In this issue of *Cell Stem Cell*, Wong et al. (2008) define a core embryonic stem cell (ESC)-like gene expression program that may be important for CSC function in multiple epithelial cancers.

Increasing evidence suggests that pathways and properties associated with normal stem cells are important for cancer development. The link between genes important for normal stem cell development and cancer is most clearly established in hematopoietic malignancies, based largely on the study of chromosomal translocations identified in leukemias and lymphomas. An extensive body of work has demonstrated that these translocations often involve genes that play critical roles in normal hematopoietic stem cell (HSC) biology (reviewed in Orkin and Zon, 2008). Human acute myelogenous

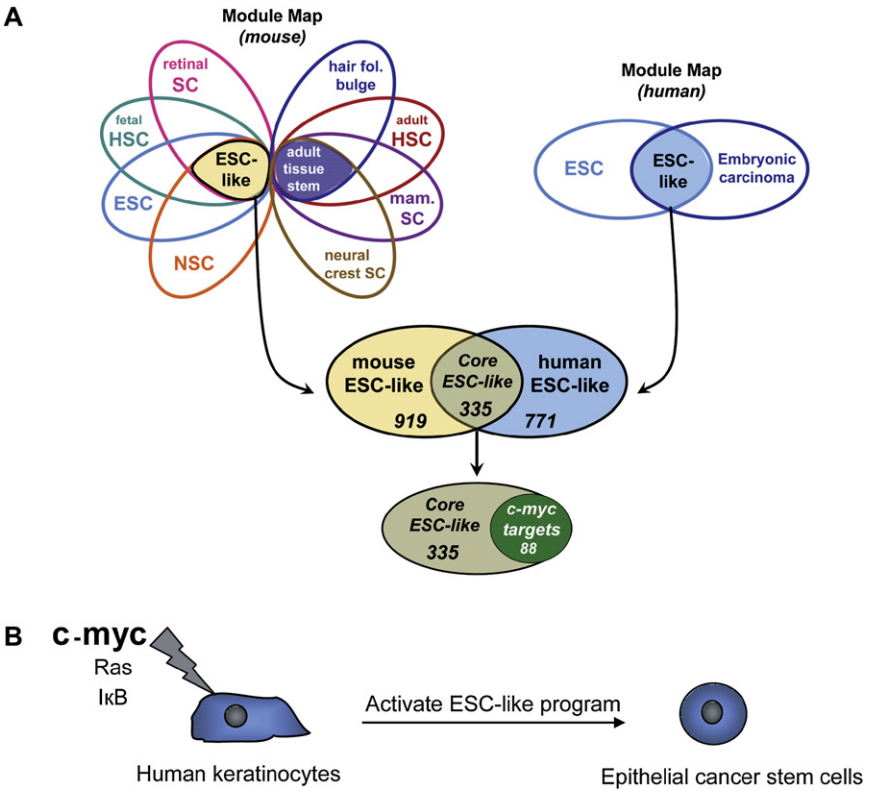

Figure 1. An ESC-like Program in Epithelial Cancer Stem Cells

(A) Certain adult tissue stem cells share a transcriptional program with ESCs, termed an ESC-like program. Comparing the mouse and human ESC-like programs reveals 335 overlapping genes. The overlapping set is used to define a "core" ESC-like program, a large subset of which has been identified as c-*Myc* targets. (B) c-*Myc* expression can activate the ESC-like program in keratinocytes and lead to the development of a tumor-forming population, possibly by inducing a cancer stem cell population.

leukemias (AML) exhibit cellular heterogeneity and harbor subpopulations (often referred to as leukemia stem/initiating cells) capable of extensive proliferation, self-renewal, and increased frequency of tumor formation, and appear to establish a developmental hierarchy similar to that observed in normal hematopoiesis (Wang and Dick, 2005). Recent effort has focused on isolating these leukemia stem cells and examining the extent to which they are similar to normal stem cells. Studies have demonstrated that certain murine and human leukemia stem cells not only are globally most similar to differentiating myeloid populations but also exhibit gene expression programs/pathways normally associated with HSCs (Jamieson et al., 2004; Krivtsov et al., 2006; Somervaille and Cleary, 2006). Thus, in some hematopoietic cancers, differentiated progeny appear to activate gene expression programs normally restricted to the stem cell population of that tissue. The extent to which stem cell programs are important in solid tumors, and in particular in tumors derived from epithelial cells, has been less clear.

The present work by Wong and colleagues (Wong et al., 2008) uses a gene module map (Segal et al., 2004) tool to assay for the presence of stem cell programs in tumors of epithelial origin (Figure 1A). The initial step in their approach was to identify gene sets that are coordinately regulated in various cell types. Some 3000 mouse gene sets were compiled from various previous studies, including gene expression profiles, ChIP-chip results, RNAi experiments, and functional annotations, such as those found in Gene Ontology databases. The gene sets were then assessed for coordinate regulation by comparing expression data obtained from mouse ESCs, adult tissue stem cells (including neural stem cells [NSCs], HSCs, retinal stem cells, neural crest stem cells, hair bulge stem cells, and mammary stem cells), and a number of differentiated cell types. Using this approach, they identified a shared subset of genes termed "ESC-like" that clustered ESCs together with NSCs, fetal liver HSCs, and retinal stem cells. Clustering away from these populations was the majority of adult tissue stem cells, including HSCs, neural crest stem cells, hair bulge stem cells, and mammary stem cells. This group shared a distinct subset of genes that were termed the "adult tissue stem" module. Interestingly, when the approach was repeated with data derived from studies of human cells, 335 genes were found to significantly overlap between mouse and human ESC-like modules. This subset was subsequently defined as the "core" ESC-like gene module.

In addition to defining the ESC-like module, the authors demonstrated that this gene expression program is activated in multiple human cancers but repressed in normal tissues. In order to study whether expression of the ESC-like module might have clinical implications for human cancers, 295 primary samples of stage I and II breast cancers and 71 stage I and II lung adenocarcinoma samples were examined. The analysis revealed a positive correlation between activation of the ESC-like module and poor tumor differentiation status, increased risk of metastasis, and a worse outcome in both sets of human tumors. The authors then examined a CSC-enriched subpopulation of breast cancer cells shown previously to express a gene expression signature that is correlated with more aggressive clinical behavior (Liu et al., 2007). The ESC-like signature was detected in the CSC-enriched CD44+CD24−/low subpopulation of human breast cancers. Thus, it appears the ESC-like module is expressed by certain human tumors and that cancer stem cells may be at least partially responsible for the presence of this signature in bulk tumors. Of note, increased expression of the ESC-like profile correlates with a worse clinical outcome, raising the possibility of its utility as a prognostic tool.

Having identified the ESC-like signature as potentially important for human tumors, the authors switched their focus to identifying potential mechanisms of signature activation. A computational screen of *cis*-regulatory motifs identified c-*Myc* as a potential regulator of the ESC-like

program. Eighty-eight of the 335 ESC-like module genes have been previously identified as c-*Myc* targets (http://www.myc-cancer-gene.org/) (Figure 1A). Strikingly, c-*Myc*, but not Src, β-catenin, E2F3, or Ras, was able to activate the ESC-like transcriptional program in adult mammary epithelial cells and primary human fibroblasts. Next, Wong et al. (2008) assessed an in vivo model of human tumor development. Consistent with results in cultured cells, c-*Myc* expression also activated the ESC-like module in tumors initiated by expression of c-Myc, Ras, and IκBα in primary human keratinocytes. Finally, the frequency of tumor initiating cells in tumors generated by the combined expression of c-Myc, Ras, and IκBα was greater than in tumors derived by expression of E2F3, Ras, and IκBα, suggesting that c-*Myc* expression promotes both activation of the ESC-like program and development of cancer stem cells in this system (Figure 1B).

The demonstration that an ESC-like program is found in multiple human cancers, and that activation of this program can be driven by c-*Myc* during conversion of primary human cells to tumor-forming populations, is particularly relevant to ongoing efforts designed to study cancer stem cell development. Growing evidence strongly supports the hypothesis that stem cell gene expression programs are activated during leukemia stem cell development. Wong et al. now provide evidence that stem cell programs are also relevant during epithelial cancer stem cell development and imply that similar mechanisms may be relevant for many cancers. An intriguing possibility that arises from the present module map analysis is that hematopoietic malignancies are a result of activation of "lineage-specific" stem cell programs, whereas epithelial tumors may be more a result of activation of ESC-like programs. This separation of hematopoietic and epithelial tumors based on "lineage-specific stem cell" programs versus the "ESC-like" module raises the question of which pattern, if any, is apparent in other malignancies, such as sarcomas. The functional significance of the activation of various stem cell pathways/programs in multiple cancer stem cell populations clearly remains to be determined.

Finally, the concept that activation of stem cell programs can influence cancer stem cell development has important implications for efforts to initiate cellular reprogramming of somatic cells into induced pluripotent stem (iPS) cells (Takahashi and Yamanaka, 2006). As discussed, c-*Myc* can activate an ESC-like program during the conversion of keratinocytes to cancer stem cells (Wong et al., 2008). However, c-*Myc* is also an important, if expendable, reprogramming factor that enhances the formation of iPS cells from fibroblasts (reviewed in Knoepfler, 2008). Together, these findings suggest that the processes of reprogramming and cancer stem cell induction may engage overlapping programs. This possibility highlights the importance of studies designed to define the transcriptional and signaling networks involved in normal and cancer stem cell development. With a better understanding of these processes, it may become possible to target cancer stem cells while not heavily impeding normal stem cell function and also to develop cellular reprogramming methods for use in regenerative medicine without undue risk of cancer stem cell development.

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