

Extra View

Stemness, cancer and cancer stem cells

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The ability of cancers to grow indefinitely has fueled the idea that cancer and stem cells may have common underlying mechanisms. Detailed gene expression maps have now shown the diversity and distinctiveness in gene expression programs associated with stemness in embryonic and adult stem cells. These maps have further revealed a shared transcriptional program in embryonic stem cells (ESC) and cancer stem cells. Surprisingly, forced activation of an ESC-like gene expression program in adult epithelial cells can reprogram them into human cancer stem cells and achieve pathologic self-renewal. The ability to create induced cancer stem cells (iCSC) may provide opportunities to better define the biology of cancer stem cells in order to trace or eliminate them in human patients.

Many organs are maintained by a small number of progenitor cells, termed stem cells, that self-renew and give rise to other cell types present in the organ.^{1,2} The unique abilities of embryonic stem cells (ESCs) and adult tissue stem cells to self-renew and give rise to multiple cell lineages have formed the basic definitions of “stemness”. Accumulating evidence suggests that cancers may have equivalent cancer stem cells, a small subset of cells that have the unique ability to initiate and perpetuate tumor growth and heterogeneity in serial transplantation experiments.¹ If cancers are truly maintained by a small population of cancer stem cells, then cancer diagnosis and therapy would be greatly improved by the ability to identify, target, and eliminate cancer stem cells. It has been theorized that a critical event in cancer initiation may be the aberrant activation of the self-renewal machinery that is normally restricted to stem cells. However, it has been unclear whether activation of a normal stem cell program is a pervasive feature or required element in common human epithelial cancers.

Recent evidence has suggested a remarkable plasticity of stem cell fates. Several types of adult tissue stem cells can be apparently reprogrammed into cell types of other lineages.³ In addition, introduction of four genes, *c-Myc*, *Oct4*, *Sox2* and *Klf4*, into mouse or human fibroblasts was sufficient to reprogram them to confer pluri-

potency indistinguishable from authentic ESCs, resulting in induced pluripotent stem cells (iPSC).⁴ These results raise the possibility that other types of stem cells, including cancer stem cells, may be experimentally created by the appropriate combination of genes.

Previous studies have attempted to define a core transcriptional program of “stemness”, but the results have been controversial. Fortunel et al. compared the shared microarray expression profiles of ESCs, neural stem cells, and hematopoietic stem cells across three independent studies, and they only found a single gene shared among the three studies.⁵ The inability to define a consensus stemness signature in a gene-by-gene analysis may suggest that different types of stem cells utilize distinct mechanisms to achieve self-renewal and pluripotency. Alternatively, the failure to identify a robust signature may be due to technical variations in stem cell isolation, degrees of cell purity, microarray platforms, or statistical analysis methods. Because intersection of gene-level analyses are more prone to noise and are inherently limited by the technically worse dataset under consideration,^{6,7} we hypothesized that a higher-order, systems-level analysis may improve the organization and classification of stem cell transcriptional programs.

Indeed, we have recently shown that embryonic and adult stem cells can be organized into two predominant groups based on their gene expression, and surprisingly, cancer stem cells may demonstrate gene expression programs similar to ESCs.⁸ We used a method termed **gene module map**⁶ to identify shared, but not necessarily universal, expression programs of ESCs and adult tissue stem cells, focusing on groups of genes, termed modules, that are supported by the consensus of multiple independent observations. For instance, we identified an ESC gene module that is comprised of significant overlap among genes that show increased expression in ESCs compared to differentiated cells, genes whose promoters are occupied by regulatory proteins conferring pluripotency such as Oct4, Nanog and Polycomb, and genes that are decreased in expression in ESCs upon depletion of these pluripotency factors.⁸ From the concordant behavior of hundreds of stem cell profiles, we find that adult tissue stem cells can be separated into two large groups, one of which shares a core transcriptional program with ESCs.⁸ These results suggest that “stemness” in different types of stem cells may not be reflected by a universal gene expression program, and that there are likely to be many routes to stemness. A recent independent large-scale analysis of stem cell transcriptional profiles also reached a similar conclusion.⁹

What might be the relationship between stemness and cancer? Using the same informatic comparison, we found that the ESC-like

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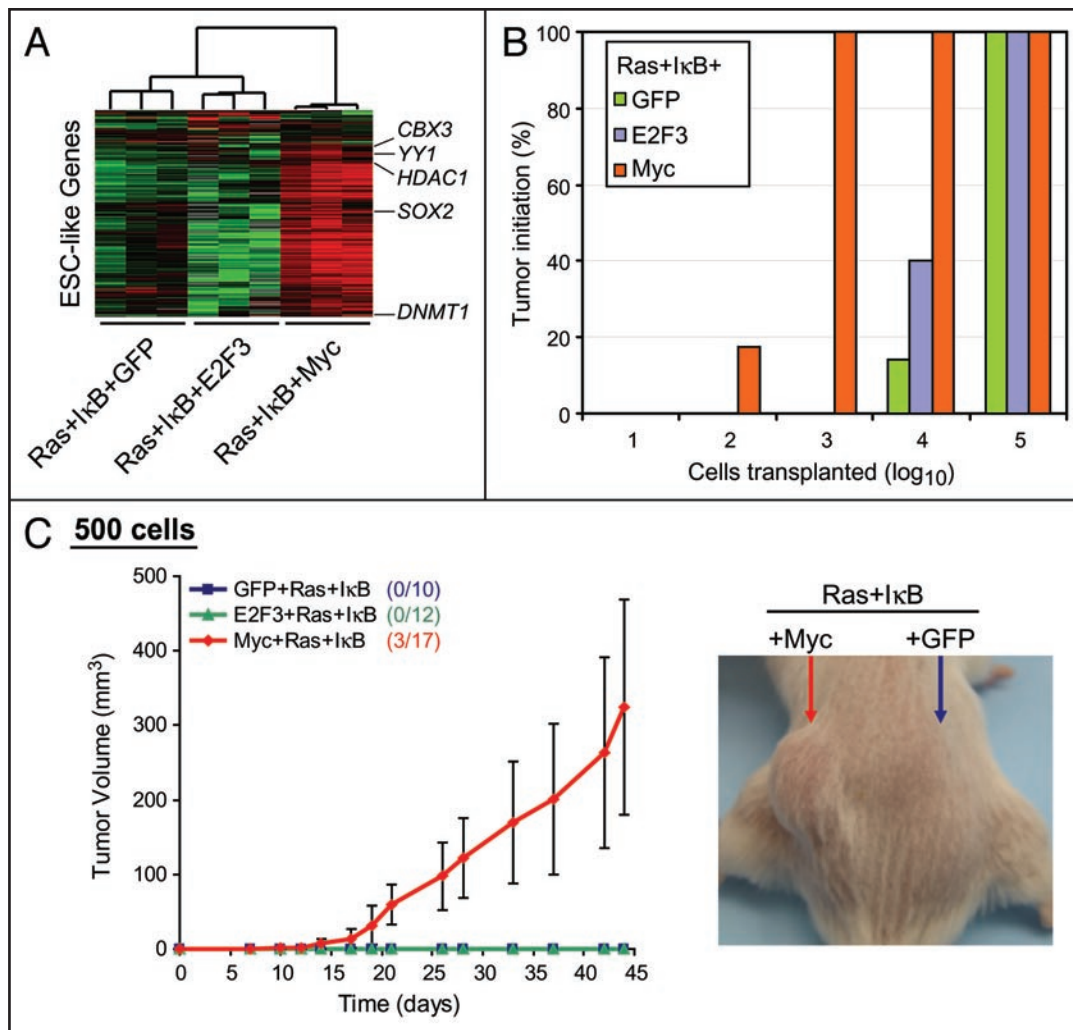


Figure 1. Creation of human cancer stem cells. (A) Identification of a genetic combination, comprised of *Ras*, *IkB* and *MYC*, that can reprogram human keratinocytes to reactivate a gene expression program resembling embryonic stem cell (ESC). (B and C) This reprogramming induces highly tumorigenic cells. Limiting dilution assays showed 150-fold increase in tumor-initiating capacity, which can serially propagate cancer formation in vivo. We predicted *MYC* to be a key driver of the ESC signature, and its role in pathologic self-renewal is distinct from cell proliferation as it cannot be substituted by *E2F3*.

transcriptional program is frequently activated in diverse types of aggressive human epithelial cancers, including cancers of the breast, liver, lung and others.⁸ Within breast and lung cancers matched by stage and other traditional criteria, higher expression level of the ESC-like gene module is a significant predictor of progression to early death.⁸ In an independent analysis of stem cell gene signatures by Weinberg and colleagues, these authors also observed increased expression of ESC-like signatures in clinically aggressive epithelial cancers, such as bladder cancer.¹⁰

To understand the origin of the ESC-like transcriptional program in human cancers, we searched for shared cis-regulatory motifs in genes that constitute the ESC module, using a computational method we developed termed **motif module map**.¹¹ We computationally identified sets of genes, termed modules, that share the presence of specific cis-regulatory motif sequences in their regulatory regions. We identified such motif modules for every single, double and three-way combination of known cis-regulatory motifs. Armed with this information, we are able to deconvolute complex transcriptional profiles, such as those from stem cells or cancers, into the candidate cis-motif and corresponding transcription factors that may

drive their transcriptional expression. Based on our motif module map, the motif for binding to the oncogene *c-Myc* was predicted to be the top driver of the ESC module.⁸ Experimental survey of multiple human oncogenes confirmed that *c-Myc* is uniquely able to activate the ESC-like transcriptional program in adult epithelial cells, which in the appropriate genetic context confers cardinal properties of human cancer stem cells in vivo (Fig. 1). In primary human adult keratinocytes transformed by *Ras* and *IkB*, *c-Myc* expression strongly induced the ESC module, and led to over 150-fold increase in tumor-initiating capacity, forming tumors with as few as 500 cells that can be serially propagated in vivo.⁸ These cells also induce unique markers of authentic cancer stem cells isolated from human patients.⁸ Thus, we term cells reprogrammed by this three gene combination “induced cancer stem cells” or iCSC.

These results have several broad implications. First, they suggest that cancer stem cells may share a surprising degree of similarity with ESCs. This finding may not be completely surprising given the use of *c-Myc* in both iPSC and iCSC, the ability of Oct4 to induce dysplasias in adult tissues, and the role of Polycomb proteins in both pluripotency and cancer progression.^{12,13} Nonetheless, our results

suggest that the ESC-like transcriptional program may be specifically reactivated in cancer stem cells. This idea poses a challenge for regenerative medicine to avoid promotion of cancer stem cells during cell-based therapy, such as in the use of iPSC. It is likely that incomplete or aberrant reprogramming to iPSC may lead to cells with some properties of iCSCs. In fact, mice derived from iPSCs frequently developed cancer.¹⁴ The close link between cancer and regeneration will no doubt be the focus of future studies in both fields.

Second, the production of iCSCs may offer a complementary route to study human cancer stem cells. Authentic cancer stem cells are rare cells in the tumor. Although they may be enriched or even purified by sorting based on multiple cell surface markers from clinical tumor specimens, the resulting cell populations may still be genetically heterogeneous (especially if derived from different patients) and too few in number for biochemical studies. The ability to trace the origin of large-scale genetic programs has enabled us to create the first human cancer stem cells in the laboratory by reactivating an ESC-like transcriptional program in adult cells. The ability to mass-produce human cancer stem cells (iCSC) is of substantial interest because it may finally give scientists the opportunity to study their origin and find drugs that may eliminate them. For the first time, it may be possible to apply powerful functional genomic methods to map chromatin states, transcription factor occupancy, and other gene regulatory events in human cancer stem cells on a genome-wide scale.

Finally, iCSCs may offer a useful comparison to ESCs and iPSCs. Self-renewal is a shared hallmark of stem cells and cancer, but important differences must exist between stem cells and cancer cells. On the one hand, such differences endow normal embryonic stem cells (ESC) with pluripotency and ability for orderly tissue differentiation; on the other hand, these differences must also endow cancer stem cells with unique pathologic features, which can be selectively targeted without harming normal tissues. iCSCs offer the ideal comparison with authentic ESC and iPSC to dissect the key differing regulatory networks in normal and pathologic stem cells. This type of dissection may identify key differences that distinguish cancer stem cells and normal stem cells; such differences should be prioritized as candidates for targeted therapies.

In 2006, Clarke and Fuller penned an influential review outlining the hypothesis connecting regeneration and cancer, which they entitled "Stem cells and cancer: Two faces of Eve".² This imagery evokes another mythological figure—Janus, the two-faced Roman God of Gates. Janus is also a symbol of transitions, whose two faces allow him to see both into the past and future. Janus may be a particularly apt symbol of the field today where rapid progress has been made in deciphering the regulatory circuitry of stem cells and reprogramming. The development of iPSC and iCSC has now opened a possible gateway into elucidating the diverse manifestations of stemness, and offers the hope of controlled regeneration and elimination of cancer stem cells.

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