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Design Principle used by Embryonic and Adult Stem Cells

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Human embryonic stem cells (ESC) are undifferentiated and are endowed with the capacities of self renewal and pluripotential differentiation. Adult stem cells (ASC) renew their own tissue, but whether they can trans-differentiate to other tissues is still debated. To understand the genetic program that underlies the functioning of stem cells, we set out to determine their gene expression profile compared with that of stem/progenitor cells from hematopoietic system (HSPC) or keratinocytes (KSPC) and their differentiation counterparts (HDC) and (KDC).We wished to determine specific genes for each stage and to analyze whether there exists a common core of so-called "stemness" genes, shared by all stem cells (SC).

We analyzed two groups of samples (Fig. 1):

(A) hematopoieitic (H) pathway;
ESC→HSPC→ HDC, and
(B) keratinocytic (K) pathway;
ESC→KSPC→KDC

The data showed that ESC express many genes at a higher level than any other cell stage and the majority of transcripts exhibited marked downregulation along the differentiation pathway.

Fig. 2 depicts the expression matrix after clustering of the genes in the H (Fig. 2A) and K (Fig. 2B) pathways. Six clusters are clearly shown. Clusters 1, 2 and 3 contain ESC genes that were down-regulated along differentiation in both H (H1-H3) and K (K1-K3) pathways. Clusters 4 and 5 contain genes that were upregulated along the differentiation pathway and clusters 6 contain genes expressed only in adult stem cells (ASC). Clearly, ESC and ASC have different gene expression profiles.

Clusters 1 and 2 contain genes that are common to ESC and ASC and therefore may represent the "stemness" genes as previously defined. It should

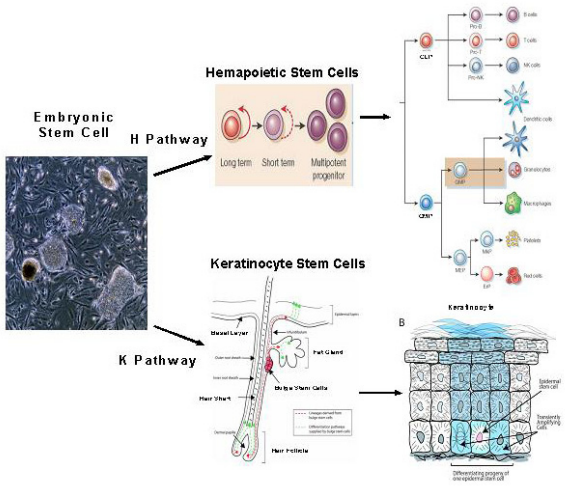

Fig. 1 *A scheme of the experimental plan to compare differentiation pathways from embryonic stem cells through stem/progenitors cells to adult differentiated cells.*

be noted, however, that many genes, well known to be markers for undifferentiated ESC or related to ESC self-renewal (e.g. *NANOG, POU5F1* (*OCT4*), *SOX2*, *FOXH1, TDGF1 (Cripto), LeftyA* & *B*, *Thy1*) belong to clusters H3 and K3 (Fig. 2), and thus are suppressed in ASC. Their roles are apparently taken over in ASC by those of clusters H6 or K6, which show expression only in ASC, and indeed contain genes known to be essential for the self-renewal of ASC, progenitors and tissue development (e.g. *TP73L (p63)*, *ITGB4* and *BNC* for skin, and e.g. *BMI1*, *CD34*, *TIE*, *KIT*, *TAL1* (*SCL*), and *RUNX1* for blood).

We looked for design principles of stem cells that accounts for both self renewal and pluripotency .A prime candidate for pluripotential differentiation is the parsimonious *"just in time"* strategy; expressing genes only when needed, i.e. at the moment of commitment to a particular differentiation path. The opposite extreme is the seemingly more

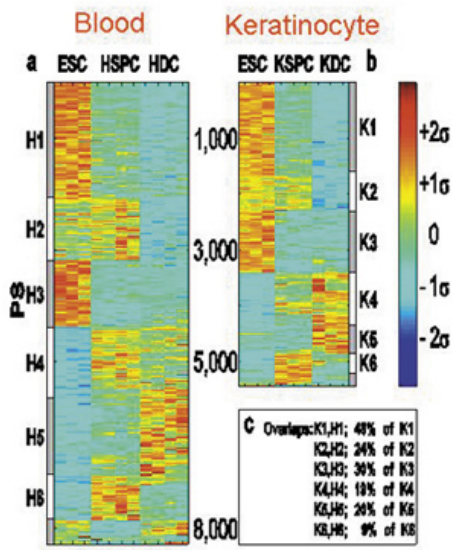

Fig. 2 *Clustering analysis of PS (Probe Sets) expression levels in hematopoietic and keratinocytic pathways. A Expression matrix of H pathway. B Expression matrix of K pathway. C Overlaps between clusters.*

wasteful *"just in case"* strategy, which keeps a wide repertoire of expressed genes, to be present in case a particular path is selected.

The clustering results showed that in the hematopoietic pathway 4392 PS (3483 genes) were downregulated and 2638 PS (1998 genes) were upregulated, while in the keratinocyte pathway 3417 PS (2758 genes) were downregulated and 1423 PS (1115 genes) were upregulated. The massive downregulation is consistent with the "*just in case*" design principle underlying pluripotential differentiation. Our data suggest that in order to maintain their potential for pluripotency, ESC "keep their options open" by promiscuous gene expression, maintaining thousands of genes at intermediate levels, to be down-regulated upon commitment to a cell fate for which they are not needed. This down-regulation is required for establishing the differentiated state.

Our analysis suggests that stem cells express a large repertoire of genes and then select a few for continued expression as they differentiate to a target tissue. Many other genes that are not needed for this tissue are knocked-down upon differentiation.

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