Stem Cells, Cancer Stem Cells
I. Leukemia stem cells
II. Stem cell transcription factors

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I. The "cancer-stem-cell hypothesis" implies that tumors are composed of a heterogeneous population of cells, within which resides a small population of cancer stem cells that are responsible for the maintenance and propagation of the tumors. These cells possess the stem cell properties of self-renewal and of inhibited differentiation, but lost the controls operating in normal stem cells, or gained mutations that endowed them with tumorigenicity. In this model, in the case of Acute myeloid leukemia (AML), the Leukemic stem cells (LSC) exhibit similar phenotype to hematopoietic stem cells (HSC) (e.g.CD34+CD38–), but also show differences from HSC. Hence LSC may represent transformed HSC or progenitor cells that acquire self-renewal by this transformation (e.g Bcr-Abl translocation in CML or Notch mutation in T-ALL).

To study the properties of AML-derived LSC we isolated the CD34+CD38- cell fraction from leukemia cells (Fig. 1) and compared their gene expression profiles to the differentiated CD34+CD38+ cells, using DNA microarrays. In spite of the heterogeneity of the LSC cell population in the five AML samples, a clear distinction was demonstrated between the LSC and their differentiated progeny. We found 409 genes that are over- or under-expressed between the two populations. Classification of the modulated genes in LSC revealed under-expression of DNA repair, signal transduction and cell cycle genes, which is consistent with the relative quiescent characteristic of stem cells, and the tendency of leukemic cells to acquire chromosomal aberrations and mutations.

We focused on the Notch ligand Jagged-2, which was found to be over-expressed in the LSC samples. We showed that DAPT, a gamma-secretase inhibitor of Notch signaling, inhibits LSC growth in colony formation assays and may inhibit the self-renewal property of leukemic stem cells. The success of leukemia therapy depends on the ability to target and eradicate the rare LSC.

II. Embryonic stem cells are endowed with two defining properties: self-renewal and pluripotency. We study the transcription factors that control these properties and try to see similarity with self- renewal of cancer cells. The transcription factor Nanog is uniquely expressed in embryonic stem (ES) cells and also in germ cell tumors and is considered important for self-renewal and pluripotency. We wanted to know if the self-renewal property of Nanog can lead to oncogenic transformation. NIH3T3 cells expressing Nanog showed an increasing growth rate and a transformed phenotype as demonstrated by foci formation (Fig. 2) and colony growth in soft agar. This suggests that Nanog and possibly other ES cell transcription factors possess an oncogenic potential that may be related to the role it plays in germ cell tumors and to its function in self renewal of ES cells.

We also studied the transcription targets of Nanog by expressing it in three cell lines, NIH3T3, H1299 and HCT116, and used microarrays to identify Nanog regulated genes.

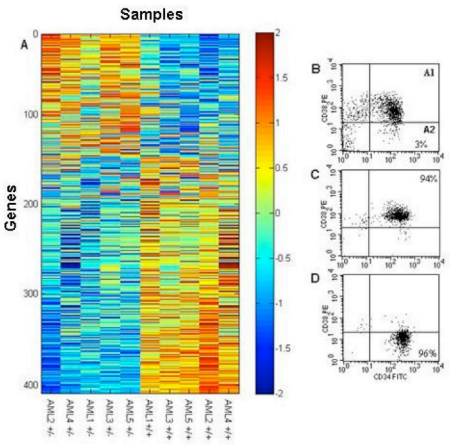

Fig. 1 *Analysis of AML stem cell modulated genes. A. Expression matrix of the modulated genes in LSC. Each row represents a single gene, and each column represents the CD34+CD38- (+/-) or the CD34+CD38+ (+/+) cell population of a particular AML patient (AML1-AML5). B. FACS analysis of AML cells, isolated from peripheral blood of AML patient 1, prior to fractionation. C. Analysis of the sorted CD34+CD38+ population. D. Analysis of the sorted CD34+CD38- population. Purity of the sorted cells is indicated by %.*

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The list of genes regulated by Nanog was unique for each cell type with only 5% overlap. More than 10% of the Nanog regulated genes, including transcription factors, are putative primary targets since their promoters were reported to bind Nanog in ES cells. Most of the genes whose promoters are bound by Nanog in ES cells, are not activated in these cell lines, suggesting that the accesibility of many genes to transcription factors may be blocked upon differentiation. The emerging picture is that in ES cells the chromatin is demethylated and accessible. Nanog, Oct4 and Sox2 bind to many promoters, most of which are suppressed to prevent differentiation. After differentiation only the cell specific genes remain accessible, as shown by the cell-restricted gene activation by Nanog.

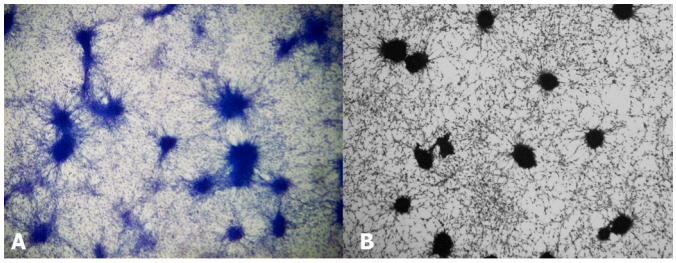

***Fig 2**. Nanog transforms NIH3T3 cells. A. Foci formation by NIH3T3 cells infected with MMLV pBabe:Nanog and . B. Foci formation by NIH3T3 infected with MMLV pBabe:HRas-v12.*

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