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ECAT: ES Cell Associated Transcripts

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Embryonic stem (ES) cells derived from inner cell mass of mammalian blastocysts grow infinitely while maintaining pluripotency. These properties have raised a hope that ES cells could be used to treat a host of degenerative diseases such as Parkinson's disease and diabetes. The hope was accelerated by the generation of pluripotent cells from human blastocysts. However human ES cells also raised substantial ethical issues since human embryos have to be destroyed to generate ES cells. One solution to avoid such ethical issues is to generate pluripotent cells directly from somatic stem and other cells. The first step toward this goal is to understand molecular mechanisms underlying pluripotency and rapid proliferation. Leukemia inhibitory factor (LIF) can maintain self-renewal of mouse ES cells through activation of STAT3. However, LIF/STAT3 is dispensable for maintenance of ICM. Furthermore, LIF is not required for self-renewal of human ES cells and several mouse ES cell lines. Mouse ES cells deficient in LIF receptor were also established. In addition, STAT3 is expressed in wide ranges of cell types and, in some cases, is essential for differentiation. These data suggest that the pathway is not fundamental for pluripotency. To search for novel factors essential for pluripotency and rapid proliferation of ES cells, we utilized *in silico* approach to identify genes that are specifically expressed in ES cells and pre-implantation embryos. We designated these genes ECAT for ES cell associated transcripts. We found that one of ECAT, encoding a novel homeoprotein NANOG, was capable of maintaining ES cell self-renewal independently of LIF/STAT3. *NANAOG*-deficient ICM failed to generate epiblast and only produced parietal endoderm-like cells. *NANOG*-null ES cells lost pluripotency and differentiated into extraembryonic endoderm lineage. These data demonstrate that NANOG is a critical factor underlying pluripotency in both ICM and ES cells. The immortality and rapid growth of ES cells make them attractive sources for stem cell therapies. However, they produce tumors (teratomas) when transplanted, which could preclude their therapeutic usage. Why ES cells, which lack chromosomal abnormalities, possess tumor-like properties is largely unknown. We found that ECAT5 encodes a novel Ras family protein ERas. We also show that human *HRasp*, which had long been recognized as a pseudogene, does not contain reported base substitutions and instead encodes the human *ERas* ortholog. ERas contains amino acid residues identical to those present in active RAS mutants and causes oncogenic transformation in NIH 3T3 cells. ERas interacts with PI3 kinase but not with Raf. *ERas*-null ES cells maintain pluripotency but show significantly reduced growth and tumorigenicity, which are rescued by cDNA expression of ERas or activated PI3 kinase. These results demonstrate that the transforming oncogene ERas plays an important role in tumor-like growth properties of ES cells. We analyzed molecular mechanisms that determine the ES cell-specific expression of the ECAT genes. We found that many of the ECAT genes are regulated by Oct4, Sox2 and Nanog. However, forced expression of these transcription factors in somatic cells failed to induce the expression of ECAT. In contrast, treatment of somatic cells with the DNA methylation inhibitor 5-aza-deoxycytidine and/or the histone deacetylase (HDAC) inhibitor tricostatin (TSA) induced the expression of many ECAT genes. Bisulfite genomic sequencing and chromatin immunoprecipitation demonstrated dynamic and unique patterns of CpG methylation and histone modification in the enhancers and promoters of the ECAT genesin ES cells, somatic cells and germ cells. These data showed that the specific expression of ECAT is determined by ES cell-specific transcription factors and epigenetic modification.